Étude biochimique sur les éléments des terres rares. III.

Sur la réaction entre la protéine et les sels du néodyme.

Par Réinosouké HARA.

(Reçu le 21 février 1949.)

Introduction. Jusqu'ici, l'action des éléments des terres rares sur la protéine a été poursuivie par M. Ajazzi-Manchini[1] (La), Sh. Kozawa[2] (La), S. Hara[3] (Ce), G. Guidi[4] (Nd), P. Niccolini[5] (Pr, Sm). De leurs études, on comprend que les éléments des terres rares généralement précipitent la protéine dans la concentration moyenne et la capacité de précipiter la protéine est très grande. J'ai employé non seulment les sels inorganiques du néodyme mais aussi les sels organiques.

Partie d'expérience.

La solution de tampon employée est $CH_3COOH-CH_3COONa$ à 1/10 N, et le pH est 5.6.

(1) La détermination de la concentration qui est nécessaire pour la précipitation.

a) L'action sur l'albumine d'oeuf.

On a dissous la protéine dans la solution de tampon, et a ajouté 1 c.c. de la solution des sels du néodyme de plusieurs concentrations à 1 c.c. de cette solution. Le résultat est montré ci-dessous.

Table 1.

sel du néodyme \ concentration du néodyme après l'addition %	4	3	2	1	1/2	1/3	1/4	1/6	1/8	1/10	1/16	1/20	1/25	1/50	1/64	1/128
$NdCl_3 \cdot 6H_2O$	—	—	—	—	++	++	+++	+++	+++	++	+	±	±	±	±	—
$Nd(NO_3)_3 \cdot 6H_2O$	—	—	—	+	+	+++	+++	+++	++	+	+	±	±	±	±	—
$Nd_2(SO_4)_3 \cdot 8H_2O$		—	++	+++	+++	+++	+++	+++	++	+	+	±	±	±	±	—
$Nd(CH_3COO)_3 \cdot H_2O$	—	—	—	+	+++	+++	+++	++	+	+	+	±	±	±	±	—
$Nd(C_2H_5COO)_3 \cdot H_2O$			—	+	+++	+++	+++	++	+	+	+	±	±	±	±	—

+++, — précipitation parfaite. ++, +, — précipitation imparfaite.
±, — louche (pas claire). —, — pas de précipité.

(1) M. Ajazzi-Manchini, *Arch. di. Fisiol*, **25** (1913), 257.
(2) Sh. Kozawa, *Biochem. Z.*, **60** (1914), 146; *Pflügers. Arch.*, **159** (1914), 449.
(3) S. Hara, *Arch. exper. Path.* **100** (1923), 222.
(4) G. Guidi, *Arch. internat. Pharmacodynamie*, **37** (1930), 307.
(5) P. Niccolini, *Arch. internat. Pharmacodynamie*, **37** (1930), 40; **40** (1931), 250.

b) L'action sur l'infusion pleurétique.

On a dissous 10 c.c. de l'infusion pleurétique dans 50 c.c. de la solution de tampon, a filtré le précipité et ajouté 1 c.c. de la solution des sels du néodyme à plusieurs concentrations à 1 c.c. de ce filtre.

Table 2.

sel du néodyme \ concentration du néodyme après l'addition %	4	3	2	1	1/2	1/3	1/4	1/6	1/8	1/10	1/16	1/20	1/25	1/50	1/64	1/128
$Nd(NO_3)_3 \cdot 6H_2O$	−	−	−	−	−	++	+++	+++	++	+	±	±	±	±	±	−
$Nd(CH_3COO)_3 \cdot H_2O$	−	−	−	−	−	+	+++	+++	+	+	±	±	±	±	±	−

c) L'action sur l'ascite.

Le même procédé à l'infusion pleurétique.

Table 3.

sel du néodyme \ concentration du néodyme après l'addition %	4	3	2	1	1/2	1/3	1/4	1/6	1/8	1/10	1/16	1/20	1/25	1/50	1/64	1/128
$Nd(NO_3)_3 \cdot 6H_2O$	−	−	−	+	++	+++	+++	+++	++	+	+	+	±	±	±	−
$Nd(CH_3COO)_3 \cdot H_2O$	−	−	−	++	+++	+++	+++	++	++	+	+	+	±	±	±	−

(2) Analyse du néodyme dans la protéine précipitée et calcul de l'équivalent du néodyme combiné avec 1 g. de la protéine. On a ajouté la solution des sels du néodyme à la solution de l'albumine d'oeuf à 0.5% (*p*H 5.6). Après trente minutes, la protéine est séparée par la centrifugation et ayant lavé cette précipitation cinq fois avec de l'eau, on l'a fait sécher dans l'exsiccateur. L'analyse du néodyme a été faite gravimétriquement comme l'oxalate. Le résultat est montré ci-dessous.

Table 4.

sel du néodyme	concentration du néodyme après l'addition. %	Nd % dans la protéine precipitée.	équivalent du néodyme combiné avec 1 g. de la protéine ($\times 10^{-5}$)
$NdCl_3 \cdot 6H_2O$	1/4	2.11	45
$Nd(NO_3)_3 \cdot 6H_2O$	1/4	2.44	52
$Nd_2(SO_4)_3 \cdot 8H_2O$	1/2	2.57	55
$Nd(CH_3COO)_3 \cdot H_2O$	1/3	2.11	47
$Nd(C_2H_5COO)_3 \cdot H_2O$	1/3	2.34	50

Conclusion. (1) Comme on a observé, les sels du néodyme se combinent très fortement avec la protéine et la font précipiter.

(2) La concentration nécessaire pour la précipitation est comme suite. a—Par la solution bien concentrée du sel, la protéine ne se précipite pas. b—La capacité de la précipitation est maximum pour la concentration moyenne.

(3) L'analyse du néodyme dans la protéine précipitée a montré que l'équivalent combiné avec 1 g. de la protéine est constant dans plusieurs sels du néodyme et sur l'albumine d'oeuf cette valeur est approximativement 50×10^{-5} (*p*H. 5.6).

(4) De ces résultats, on peut dire que l'absorption et l'évacuation sont assez faibles parce que la formation des composés insolubles des sels du néodyme et de la protéine conduit à se déposer dans le système réticulendothérial.

En terminant ce compte rendu de mes recherches, j'ai à exprimer mes sincères remerciments à M. le Prof. Kenjiro Kimura pour les aimables et très utiles indications, ainsi qu' à M. M les Profs. Kéigi Misawa, Taku Uémura, Eiji Ochiai et à Yosoji Ito, et à M. M les Profs. adjoint Kazuo Kuroda et Taro Kawamura, qui ont bien voulu me prêter leurs bienveillants conseils pour mes expériences.

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Fundamental Studies on the Current-Voltage Curve in Polarography. II.*

Derivation of the Activation-Controlled Polarographic Waves of Simple Metal Ions from the Standpoint of Chemical Kinetics.

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In the previous paper[1] the authors derived the formula of the reversible current-voltage curve from the standpoint of chemical kinetics,

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(1) N. Tanaka and R. Tamamushi, This Bulletin, 22 (1949), 187.

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and showed that the polarographic electrode potential can be defined by the Nernst's equation if the rate-determining step of the net electrode process is diffusion. In the present paper the authors analyse the relation between the current flowing and the electrode potential, when the rate-determining step is the activation process at the potential region where the rates of the forward and reverse reactions are nearly the same. By the same treatment as in the case of diffusion-control, some interesting results for the current-voltage relation are obtained. The theoretical curve which is analogous to the usual irreversible current-voltage curve is obtained under these conditions, and the meaning of the half-wave potential of such a wave is set down.

The Derivation of Equations.

Consider the net electrode process which contains the diffusion process and the electron-transfer reaction (the activation process) only. As the oxidation-reduction process, the reaction $M^{m+} + n\varepsilon \rightleftarrows M^{(m-n)+}$ is considered, in which M^{m+} and $M^{(m-n)+}$ symbolize the oxidant and the reductant, respectively. Let us assume that the reduction is the forward reaction and the oxidation is the reverse. In the absence of any external potential, the rates of the two reactions are represented as follows:

$$\begin{aligned} &\text{forward:} && s\frac{kT}{h}e^{-\Delta F_1/RT} \equiv \kappa_1 \\ &\text{reverse:} && s\frac{kT}{h}e^{-\Delta F_2/RT} \equiv \kappa_2 , \end{aligned} \qquad (1)$$

where s is the constant concerning the nature of the electrode surface, ΔF_1 and ΔF_2 are the free energies of activation and κ_1 and κ_2 are the abbreviations of the corresponding terms, respectively. If the particular potential V is applied to the electrode against the interior of the solution, the two rates must be written as follows:

$$\begin{aligned} &\text{forward:} && \kappa_1 e^{-\frac{n\alpha VF}{RT}} \equiv \kappa_1 E_1 \\ &\text{reverse:} && \kappa_2 e^{\frac{n(1-\alpha)VF}{RT}} \equiv \kappa_2 E_2 \end{aligned} \qquad (2)$$

αV is the fraction of the total applied potential V which will promote the forward reaction, and therefore $(1-\alpha)V$ will retard the reverse. If the activities of the oxidant and the reductant at the electrode surface are represented by $[A_o]$ and $[A_R]$, the current flowing by the electrode activation process is expressed by equation (3).

$$I = n\varepsilon\{\kappa_1 E_1[A_o] - \kappa_2 E_2[A_R]\} \qquad (3)$$

This current is carried by the diffusion process of oxidant, namely:

$$I = n\varepsilon K_O([C_O]^\circ - [C_O]) = n\varepsilon \frac{K_O}{f_O}([A_O]^\circ - [A_O]) = n\varepsilon K_O'([A_O]^\circ - [A_O]) \quad (4)$$

or by that of reductant, namely:

$$I = n\varepsilon K_R([C_R] - [C_R]^\circ) = n\varepsilon \frac{K_R}{f_R}([A_R] - [A_R]^\circ) = n\varepsilon K_R'([A_R] - [A_R]^\circ) \quad (5)$$

where $[A_O]^\circ$, $[A_R]^\circ$ are the activities of the oxidant and reductant in the body of the solution, $[C_O]$, $[C_R]$, $[C_O]^\circ$, $[C_R]^\circ$ are the concentrations of the oxidant and reductant at the electrode surface and in the body of the solution, and f_O, f_R symbolize the activity-coefficients of the corresponding terms. From equations (3), (4) and (5) the general relation between the current I and the electrode potential V is obtained:

$$I = n\varepsilon \frac{K_O' K_R' (\kappa_1 E_1 [A_O]^\circ - \kappa_2 E_2 [A_R]^\circ)}{\kappa_1 E_1 K_R' + \kappa_2 E_2 K_O' + K_O' K_R'} = n\varepsilon \frac{[A_O]^\circ - [A_R]^\circ \dfrac{\kappa_2 E_2}{\kappa_1 E_1}}{\dfrac{1}{K_O'} + \dfrac{1}{\kappa_1 E_1} + \dfrac{1}{K_R'} \dfrac{\kappa_2 E_2}{\kappa_1 E_1}} \quad (6)$$

$$= n\varepsilon \frac{[A_O]^\circ \dfrac{\kappa_1 E_1}{\kappa_2 E_2} - [A_R]^\circ}{\dfrac{1}{K_O'} \dfrac{\kappa_1 E_1}{\kappa_2 E_2} + \dfrac{1}{\kappa_2 E_2} + \dfrac{1}{K_R'}} \quad (6')$$

Now let us consider the magitudes of K_O', K_R', $\kappa_1 E_1$ and $\kappa_2 E_2$ in equations (6) and (6'). K_O' and K_R', which depend on the diffusion coefficients of the oxidant and the reductant in the solution, may be generally regarded to be almost identical. On the other hand, $\kappa_1 E_1$ and $\kappa_2 E_2$ are the functions of the electrode potential V that is continuously changed in polarography. Therefore, the magnitudes of the above four values should be compared at the particular potential region separately. So, in the present treatment, the total current-voltage curve is divided into three parts according to the value of V, i. e., $\kappa_1 E_1 \cong \kappa_2 E_2$ (Part A), $\kappa_1 E_1 > \kappa_2 E_2$ (Part B) and $\kappa_1 E_1 < \kappa_2 E_2$ (Part C).

Part A of the Current-Voltage Curve. If it is assumed that V equals V_0 when $\kappa_1 E_1$ just equals $\kappa_2 E_2$; V is nearly equal to V_0 in this potential region. From the condition for the activation-controlled reaction, the following relation is satisfied in this part, i. e.,

$$\kappa_1 E_1 \cong \kappa_2 E_2 \ll K_O', K_R'. \quad (7)$$

Applying this relation to equation (6) or (6'), the approximate equation of the current-voltage curve in this region can be obtained:

$$I/n\varepsilon = \kappa_1 E_1[A_O]^\circ - \kappa_2 E_2[A_R]^\circ \qquad (8)$$

From this formula it can be seen that the current flowing at such an electrode potential is determined only by the rates of activation process.

Part B of the Current-Voltage Curve. In this potential region, the more negative the electrode potential V, the greater is the rate of the forward reaction $\kappa_1 E_1$, while the rate of the reverse reaction $\kappa_2 E_2$ tends to be negligibly small.

(a). $\kappa_1 E_1$ becomes almost equal to both of K'_O and K'_R.—Then, $\kappa_2 E_2/\kappa_1 E_1$ is very small and nearly equal to zero. Therefore, considering these relations, the equation for the current-voltage curve of this region can be derived from equation (6), namely:

$$I/n\varepsilon = \frac{[A_O]^\circ}{\frac{1}{K'_O} + \frac{1}{\kappa_1 E_1}} = \frac{K'_O \kappa_1 E_1 [A_O]^\circ}{K'_O + \kappa_1 E_1} \qquad (9)$$

(b). $\kappa_1 E_1$ is greater than both of K'_O and K'_R.—In this case, obviously, $\kappa_2 E_2/\kappa_1 E_1$ tends to zero. Hence, from equation (6) the equation

$$I = n\varepsilon K'_O[A_O]^\circ = n\varepsilon K_O[C_O]^\circ \equiv (I_d)_O \qquad (10)$$

is obtained, which represents the limiting case and corresponds to the limiting current in polarography.

From equations (9) and (10), it can be said that at Part B-(a) the current is dependent on the characters of the oxidant, but it is independent of those of the reductant, and that Part B-(a) corresponds to the reduction wave of the polarographic wave. Furthermore, the limiting current of such a case is expected to be defined by the Ilkovič's equation.

Part C of the Current-Voltage Curve. In this region the rate of the reverse reaction increases as the electrode potential V is going to be more positive than V_0, while the rate of the forward reaction decreases to a negligibly small value. By the same treatment as for Part B, the equations for the anodic wave and the anodic limiting current are obtained from equation (6′) as follows:

(a). $\kappa_2 E_2$ becomes almost equal to both of K'_O and K'_R.

$$I/n\varepsilon = -\frac{[A_R]^\circ}{\frac{1}{K'_R} + \frac{1}{\kappa_2 E_2}} = -\frac{K'_R \kappa_2 E_2 [A_R]^\circ}{K'_R + \kappa_2 E_2} \qquad (11)$$

(b). $\kappa_2 E_2$ is greater than both of K'_O and K'_R.

$$I = -n\varepsilon K'_R[A_R]^\circ = -n\varepsilon K_R[C_R]^\circ \equiv (I_d)_R \qquad (12)$$

These equations (8), (9), (10), (11) and (12) are expected to account for the total wave of oxidation-reduction process which proceeds at the polarographic electrode under the conditions above described.

Theoretical Analysis of the Curve.

In the following, the meaning of the half-wave potential, the tangent of the curve and the other important properties of the curve will be described in relation to the equations derived above.

The Electrode Potential where the Current is Equal to Zero. In Part A of the current-voltage curve there must be such a point where the current flowing is zero, i. e., $I = 0$. From equation (8) the relation between the electrode potential and the activities of the oxidant and the reductant in the body of the solution can be easily obtained as follows:

$$\left[\frac{\kappa_2 E_2}{\kappa_1 E_1}\right]_{I=0} = \frac{[A_O]^\circ}{[A_R]^\circ} \tag{13}$$

or

$$[e^{n(V-V_0)F/RT}]_{I=0} = \frac{[A_O]^\circ}{[A_R]^\circ}$$

in which the condition, $I = 0$, is satisfied. That is to say, the potential V, under the condition $I = 0$, may be determined only by the activities $[A_O]^\circ$ and $[A_R]^\circ$, and, furthermore, if $[A_O]^\circ$ is equal to $[A_R]^\circ$, this potential will represent the normal oxidation-reduction potential of the system. Therefore, if $[A_O]^\circ$ and $[A_R]^\circ$ are known and the potential $[V]_{I=0}$ are determined from the experimental curve, the normal oxidation-reduction potential V_0 can be obtained from the experimental result. In practice, since, in this potential region, the current flowing is usually very small and the inclination of the curve at the point $[V]_{I=0}$ is also very small as shown later, it will be somewhat difficult to determine the correct value of V_0 by this method.

Half-Wave Potential. There are two half-wave potentials in the total current-voltage curve, i. e., the half-wave potentials of the cathodic part and the anodic part. First, the relations for the cathodic half-wave potential will be derived, and then for the anodic one in the same way.

By substituting $(I_d)_O$ for $n\varepsilon K_O'[A_O]^\circ$ of equation (9), the equation,

$$\kappa_1 E_1 = \frac{K_O' I}{(I_d)_O - I}$$

or

$$V = -\frac{RT}{n\alpha F}\left(\frac{\Delta F_1}{RT} + \ln\frac{h}{kT}K_O' + \ln\frac{I}{(I_d)_O - I}\right), \tag{14}$$

is obtained. At the half-wave potential the current flowing is one half of the limiting current $(I_d)_o$, namely:

$$I = \frac{(I_d)_o}{2} \quad . \tag{15}$$

From equations (14) and (15) the condition for the cathodic half-wave potential is derived as follows:

$$[\kappa_1 E_1]_{\frac{(I_d)_o}{2}} = K_o'$$

or

$$[V]_{\frac{(I_d)_o}{2}} = -\frac{RT}{naF}\left(\frac{\Delta F_1}{RT} + \ln \frac{h}{kT} K_o'\right) \quad . \tag{16}$$

The equation (16) shows that the half-wave potential depends on the constant K_o', and that if the value of α is independent of the activity of the oxidant, it will be the particular constant for the oxidant concerned.

As for the anodic half-wave potential analogous equations can be obtained, i. e.,

$$[\kappa_2 E_2]_{\frac{(I_d)_R}{2}} = K_R'$$

or

$$[V]_{\frac{(I_d)_R}{2}} = \frac{RT}{n(1-\alpha)F}\left(\frac{\Delta F_2}{RT} + \ln \frac{h}{kT} K_R'\right) \quad . \tag{17}$$

The Relation between $\log(I/I_d - I)$ and V. From equations (14) and (17) it is obvious that the relation between $\log(I/I_d - I)$ and V must be linear and the absolute value of the tangent of this line is $RT/n\alpha F$ or $RT/n(1-\alpha)F$ according to the cathodic or anodic part. If this relation is plotted and the tangent of the line is determined, the value of α can be calculated. Although many investigators have often used this linear relation for the criterion of reversibility of polarographic wave, it is obvious that this may hold also in such a case when the electrode potential is not defined by the Nernst's equation. In the case when the oxidation-reduction reaction concerned is uni-valent, if the wave is reversible, the theoretical inclination of the above relation must be 0.059 volt at 25°C, hence, if the experimental result deviates from this value, we may conclude that the electrode activation process does not exist in an equilibrium state.

Index Potential $V_i^{(2)}$. In the potentiometric study of the oxidation-reduction systems the term "index potential" has been used to specify the types of the titration curve. A similar quantity has been applied

(2) L. Michaelis and M. P. Schubert, *Chem. Rev.*, **22** (1938), 437.

to analyse the type of polarographic wave. As the index potential V_i of the polarographic wave the quantity,

$$V_i = [V]_{\frac{3I_d}{4}} - [V]_{\frac{I_d}{2}} \quad \text{or} \quad [V]_{\frac{I_d}{2}} - [V]_{\frac{I_d}{4}}{}^{(3)} \tag{18}$$

is given, where $[V]_{\frac{3I_d}{4}}$ and $[V]_{\frac{I_d}{4}}$ are the potentials at which the wave-heights are $3I_d/4$ and $I_d/4$ respectively. From equation (9) the condition for $[V]_{\frac{I_d}{4}}$ of the cathodic part is to be

$$[\kappa_1 E_1]_{\frac{I_d}{4}} = \frac{K_O'}{3}$$

or

$$[V]_{\frac{I_d}{4}} = -\frac{\Delta F_1}{naF} - \frac{RT}{naF}\ln\left(\frac{h}{kT}\frac{K_O'}{3}\right) . \tag{19}$$

Substracting equation (19) from equation (16) and rearranging, equation (20) is obtained, by which the value of a can be calculated.

$$a = \frac{RT}{nF}\frac{\ln 3}{[V]_{\frac{I_d}{4}} - [V]_{\frac{I_d}{2}}} = \frac{0.059}{n}\frac{0.477}{[V]_{\frac{I_d}{4}} - [V]_{\frac{I_d}{2}}} \tag{20}$$

This method of calculation of a is essentially identical with the method described above. However, the accuracy of this method is lower than the previous method, because equation (20) utilizes only two particular points of the experimental curve, while the previous method uses a series of points of the curve to determine a.

First Derivative of the Current-Voltage Relation.—Tangent of the Curve. Differentiating the equations (8), (9) and (11) with respect to the electrode potential V, the tangents of the curves of Parts A, B and C are represented as follows:

Part A
$$\frac{dI}{dV} = -n\varepsilon\frac{nF}{RT}\{a[A_O]^\circ\kappa_1 E_1 + (1-a)[A_R]^\circ\kappa_2 E_2\} \tag{21}$$

Part B
$$\frac{dI}{dV} = -n\varepsilon\frac{nF}{RT}aK_O'\kappa_1 E_1\frac{K_O'[A_O]^\circ}{(K_O' + \kappa_1 E_1)^2} \tag{22}$$

Part C
$$\frac{dI}{dV} = -n\varepsilon\frac{nF}{RT}(1-a)K_R'\kappa_2 E_2\frac{K_R'[A_R]^\circ}{(K_R' + \kappa_2 E_2)^2} \tag{23}$$

From equations (13) and (21) the tangent of the curve at the point $I = 0$ can be obtained.

(3) R. Brdička, *Z. Electrochem.*, **47** (1941), 314.

$$\left[\frac{dI}{dV}\right]_{I=0} = -n\varepsilon\frac{nF}{RT}[A_O]^\circ\kappa_1 E_1 \tag{24}$$

The tangent at the cathodic half-wave potential is derived by the application of equation (16) to equation (22):

$$\left[\frac{dI}{dV}\right]_{(I_d)_O/2} = -n\varepsilon\frac{nF}{4RT}a\kappa_1 E_1[A_O]^\circ = -n\varepsilon\frac{naF}{4RT}K_O'[A_O]^\circ \tag{25}$$

Now let us compare the two tangents (24) and (25) with each other. Dividing (25) by (24), equation (26) is obtained as follows, unless the value of a is very small:

$$\frac{\left[\frac{dI}{dV}\right]_{(I_d)_O/2}}{\left[\frac{dI}{dV}\right]_{I=0}} = \frac{aK_O'}{4[\kappa_1 E_1]_{I=0}} > 1 \ . \tag{26}$$

From the result, it can be said that the cathodic wave is far steeper than the wave of part A. The same relations are to be held with between the anodic wave and the wave of Part A.

Second Derivatives of the Current-Voltage Relation.—Inflection Point of the Curve. By deriving the second derivatives of equations (8), (9) and (11) with respect to the electrode potential V and by making these derivatives equal to zero, the inflection points of the current-voltage curves are obtained. The three following relations are obtained for the conditions of the inflection points of the waves, i. e.,

Part A
$$\left[\frac{\kappa_2 E_2}{\kappa_1 E_1}\right]_{\text{infl. pt. A.}} = \frac{a^2}{(1-a)^2}\frac{[A_O]^\circ}{[A_R]^\circ} \tag{27}$$

Part B
$$[\kappa_1 E_1]_{\text{infl. pt. B.}} = K_O' \tag{28}$$

Part C
$$[\kappa_2 E_2]_{\text{infl. pt. C.}} = K_R' \tag{29}$$

It is pointed out from these equations that the inflection point of cathodic or anodic wave is identical with the corresponding half-wave potential. Consequently it is concluded that there may be three inflection points in the total region of the polarographic oxidation-reduction wave.

The Tracing of the Theoretical Curve Using the Equations Derived Above. As an example, let us assume some hypothetical values of ΔF_1, ΔF_2, a, $[A_O]^\circ$, $[A_R]^\circ$, K_O' and K_R' as follows, and trace the theoretical curve according to the obtained equations (8), (9) and (11) (Fig. 1).

$\Delta F_1 = 15.0 \times 10^4$ joule, $\Delta F_2 = 13.1 \times 10^4$ joule,
$[A_O]^\circ = [A_R]^\circ = 1.04 \times 10^{-3}$ mol/l., $K'_O = K'_R = 1.8 \times 10^{-8}$ amp./mol/l.,
$a = 0.6$, $n = 1$.

Under these conditions the following values are obtained for $[V]_{\frac{(I_d)_O}{2}}$, $[V]_{\frac{(I_d)_R}{2}}$ and $[V]_{I=0}$, i. e.,

$[V]_{\frac{(I_d)_O}{2}} = -0.569$ volt vs. hydrogen electrode.
$[V]_{\frac{(I_d)_R}{2}} = +0.353$,, ,, ,, ,,
$[V]_{I=0} = -0.200$,, ,, ,, ,,

From Fig. 1 it can be said that the theoretical curve is essentially the same, at least qualitatively, as some types of irreversible waves obtained in polarography, and that the equations cited above will account for the natures of the polarographic waves which have not yet been interpreted.

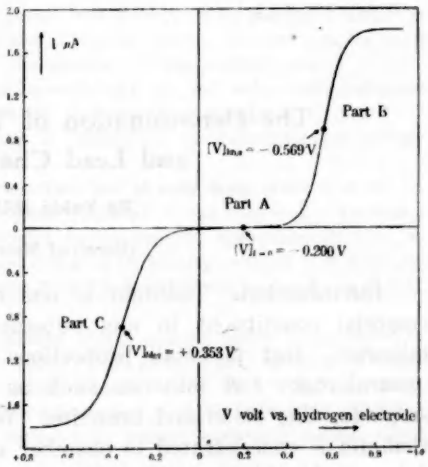

Fig. 1. Theoretical Curve.

Summary.

In this paper the authors have derived the equations of the polarographic current-voltage curve from the standpoint of chemical kinetics, assuming that the net electrode process is activation-controlled. The results obtained are summarized as follows:

(1) Half-wave potential is the constant of the substance concerned, and is independent of its activity.

(2) The potential where the current is equal to zero is only defined by the activities of the oxidant and the reductant in the body of the solution.

(3) The inflection point of the cathodic or anodic wave agrees with its half-wave potential.

(4) The relation between $\log (I/I_d - I)$ and V of the cathodic or anodic wave is a straight line.

(5) The values of ΔF_1, ΔF_2 and a are possible to be calculated from the experimental data.

(6) The theoretical curve is essentially the same as the experimental curve of the irreversible type.

The authors take this opportunity of acknowledging their gratitude to Director San-ichiro Mizushima for his kind advice and encouragement throughout this study. The cost of this research has been defrayed from the Scientific Research Encouragement Grant from the Department of Education, to which the authors' thanks are due.

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The Determination of Thallium in Flue-Dust and Lead Chamber Muds.

By Yukio MURAKAMI.

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Introduction. Thallium is one of the rare elements found as an essential constituent in any substantial quantity in only two or three minerals. But in small proportions thallium is widely distributed in natural rocks and minerals such as carnalite, alunite, copper and iron sulphide, zinc blend and braunite. When they are used as in industries, thallium is concentrated in chamber muds and flue-dust giving rise to a determinable amount. Thus thallium is more usually found accumulated in these substances rather than as a constituent of minerals.(1)

Thallium was discovered spectroscopically in chamber muds by Crookes and Lamy(2) independently in 1861. Since then various workers had succeeded in determining the thallium content in both chamber muds and flue-dust.

When the determination of thallium content in such samples was undertaken for the purpose of securing the most abundant technical source of the element, the author reviewed the methods applied hitherto. But they were found to be unsatisfactory in some respects from the reason that they require an elaborate process of separation due to the

(1) W. T. Roepper, *Am. Jour. Science*, (2), **35** (1863), 421; R. Böttger, *Lieb. Ann.*, **126** (1863), 177, 266; F. Wöhler, *Lieb. Ann.*, **142** (1867), 263; T. Carstanjen, *J. Prakt. Chem.*, **102** (1867), 65: N. N. Melnikow, *Uspeki. Chim.*, **4** (1935), 256; A. V. Petar, *U. S. Bur. Mines Information*, circular No. **6453** (1931), 31.

(2) W. Crookes, *Chem. N.*, **3** (1861), 193, 303; *J. Chem. Soc.*, **17** (1864), 115; *Chem. N.*, **7** (1863), 133; A. Lamy, *Compt. rend.*, **54** (1862), 1255; *Lieb. Ann.*, **124** (1862), 215.

presence of many metals, such as copper, iron, zinc and lead, etc., occurring in quantities hundreds and thousands times greater than thallium.

However, among these methods proposed up to the present date the one suggested by Spacu and Pope[3] appeared to be the most effective and an improvement upon it was successfully undertaken by the author as was already described in detail in a previous report.[4]

Thus a minute amount of thallium as slight as 0.001% can be determined by this improved method with fairly good results. The results obtained from about 45 samples of chamber muds and flue-dust collected from sulfuric acid plants and refineries in Japan are described in this report.

Experiment. (a) Moisture: Moisture content was determined by placing a sample in original state in an air oven for 2 hours and drying at 105°C. Several samples were hygroscopic after drying, probably owing to the presence of free sulfuric acid.

(b) Color: The color of a sample was compared with the soil color table published by Japan Forestry Society in accordance with the National Color Standard.

(c) Specific gravity: The specific gravity is determined by measuring the sample already dried (105°C) with a pycnometer.

(d) Thallium determination: This determination has already been given in detail by the author in a previous report but will be briefly recorded in the following. According to the amount of thallium content to be expected in the sample, approximately 30 g. to 50 g. of the dried powdered sample is taken and dissolved by heating with an acid mixture, consisting of one part of H_2O, two parts each of HCl and HNO_3 and four parts of H_2SO_4, being mixed in the order named.

Heating is continued until all traces of copious sulfur dioxide fumes have ceased to appear. The residual cake is disintegrated with 1 N hot sulfuric acid solution, to which it is preferable that a few drops of H_2O_2 is added to reduce any Tl^{+++} that may have been formed in the process. After standing for a few minutes this is thoroughly extracted and neutralized first with sodium hydroxide followed by sodium carbonate solution until carbon dioxide has not been evolved.

Next, sulfur dioxide is passed through the solution until it cools. After filtration the filtrate is heated to boiling to expel sulfur dioxide and acidified to about 1 N hydrochloric acid. Particles of potassium chlorate are added and then 0.5 g. of ammonium chloride, the addition of which is effective in determining a minute amount of thallium. Heating is continued until the filtrated solution is boiled down to half its bulk.

If saturated cobalti-hexammine trichloride solution is added after cooling and filtering, a heavy orange yellow precipitate of $Co(NH_3)_6(TlCl_6)$ will instantaneously appear.

This precipitate is pure enough to be weighed on a glass filter (G4, for fine precipitate), assured to constant weight, after it has been washed thoroughly with 2% HCl solution followed by three times each of 5 ml. 95% alcohol and 5 ml. ether and dried at 105°C. Thallium content is calculated from the following equation:

$$\text{Tl}\ (\%) = \frac{\text{Weight of precipitate} \times 0.35346}{\text{Weight of sample taken}} \times 100\,(\%)$$

(e) Reproducibility: The spectroscopic analysis shows that flue-dust and chamber muds contain a large amount of diverse metals, for example, such as the following:

No. 1. Zn, Cu, Pb, Fe, As, K (major), Ca, Al, Na, Si, Ti, Bi, Mg, Tl, Ge, Cd, Sb, Ag, Ni, Mo (minor).

(3) G. Spacu and A. Pope, *Z. anal. Chem.*, **102** (1940), 322.
(4) Y. Murakami. *This Bulletin*, **22** (1949), 206.

No. 2. Fe, Zn, Ca, Al, Pb, Sb, Mg, Si, Cu, As (major), Bi, Tl, Ba, Sn, K, Na, Sb, Si, In, Ge, Ag, Ti, Co, Cd, Mn.

No. 3. Zn, Pb, Sn, K, Na, Sb, As (major), Tl, Fe, In, Ca, Al, Ag, Cu, Cd, Mo, Mg, Bi, Si.

The interference of these metals in the determination of thallium had been thoroughly tested as already reported and no fatal interference has been observed. To ascertain the reproducibility of this method, three main samples of flue-dust were selected for this test upon reference to the results obtained from the spectroscopic test and a known amount of thallium was added to the samples. Thallium was determined by the newly proposed method and the result is shown in Table 1.

Table 1.
Reproducibility

Sample	Thallium added (g.)	Thallium complex (g.)	Thallium found (g.)	Thallium calculated (g.)	Difference (g.)
1. Flue-dust abundant in copper	$\triangle\overline{0.01149}$	0.0202 0.0530	0.00714 0.01873	$\triangle\overline{0.01863}$	$\triangle\overline{+0.00010}$
2. Flue-dust abundant in iron	$\triangle\overline{0.01149}$	0.0255 0.0581	0.00901 0.02054	$\triangle\overline{0.02050}$	$\triangle\overline{+0.00004}$
3. Flue-dust abundant both in copper and iron	$\triangle\overline{9.01149}$	0.0171 0.0497	0 00604 0.01761	$\triangle\overline{0.01753}$	$\triangle\overline{+0.00008}$

Complete satisfaction was obtained from this method: first, it is not hazardous owing to the unnecessity of a preliminary separation of metals: secondly, it is most successfully effective in the determination of small quantities of thallium as little as 0.01%: and thirdly, the results obtained show a minimum average deviation, being less than 1%.

Results. The object was to determine the minute amounts of thallium in 46 samples of flue-dust and chamber muds collected from sulfuric acid plants, fertilizer factories and refineries in Japan. The results are shown in Table 2.

Table 2.
Thallium content in flue-dust and chamber muds

No.	Company	Factory	Moisture (%)	Sp. gr.	Thallium content (%)	Remarks
1	Dainippon Kogyo	Hasei Kogyo	10.44	4.65	0.105	
2	Sumitomo Met. Ind. Co., Ltd.	Gensan Factory	1.23	0.70	0.089	Cottrell dust
3	Nitto Chem. Ind. Co., Ltd.	Yokohama Factory	4.54	3.39	0.042	Cottrell dust
4	Konoshima Chem. Ind. Co., Ltd.	Konoshima Factory	4.04	3.44	0.120	
5	Nippon Soda Ind. Co., Ltd.	Aizu Factory	3.59	3.47	0.016	Cottrell dust
6	Asahi Chem. Ind. K.K.	——	0.52	2.09	0.025	
7	Nippon Kogyo K.K.	Saganoseki Refinery	3.87	2.10	0.169	Cottrell dust

Table 2. (*continued.*)

No.	Company	Factory	Moisture (%)	Sp. gr.	Thallium content (%)	Remarks
8	Nitto Ryuso K.K.	Tokyo Factory	6.84	3.18	0.021	
9	Toa Kagaku Seiren K.K.	Osaka Factory	9.97	3.12	0.038	Cottrell dust
10	Nippon Jukagaku K.K.	——	2.97	3.08	0.000	
11	Nippon Hydrogen Ind. Co., Ltd.	Onahama Factory	8.14	2.86	0.115	Cottrell dust
12	Nippon Kogyo K.K.	Toyama Factory	3.05	3.05	0.227	Cottrell dust
13	Showa Denko K.K.	Kawasaki Factory	9.61	3.57	0.281	Sulfide ore
14	Nitto Ryuso K.K.	Niigata Factory	7.62	2.98	0.000	
15	Niigata Sulfuric Acid Co., Ltd.	Niigata Factory	9.15	4.15	0.029	Cottrell dust
16	Sansei Kogyo K.K.	Choko Refinery	2.38	4.49	0.031	Chimney dust
17	Teikoku Kohatsu K.K.	Murakami Refinery	1.12	5.33	0.028	Dust chamber
18	Toa Kogyo K.K.	Osaka Factory	5.58	3.54	0.059	
19	Ishihara Kogyo K.K.	Yokkaichi Factory	13.92	5.98	0.010	Smelting furnace
20	Nippon Kogyo K.K.	Chinnanbo Refinery	1.37	0.80	0.074	
21	Nippon Kogyo K.K.	Nagoya Factory	6.76	3.42	0.062	Sulfide ore
23	Furukawa Min. Ind. Co., Ltd.	Nagamatsu Factory	1.67	1.26	0.020	Chamber muds
25	Nippon Kogyo K.K.	Ouji Factory	3.48	3.46	0.006	Sulfide ore (Osaruzawa)
26	Nippon Kogyo K.K.	Ouji Factory	7.43	3.58	0.040	Sulfide ore (Hitachi)
27	Nippon Kogyo K.K.	Ouji Factory	5.23	2.87	0.010	Sulfide ore (Hitachi and Matsuo)
28	Nippon Kogyo K.K.	Chinnanbe Refinery	1.38	0.71	0.012	Hourin mine
29	Mitsui Min. Ind. Co., Ltd.	Nihi Refinery	4.70	1.21	0.091	
30	Sumitomo Min. Ind. Co., Ltd.	Kunitomi Refinery	8.11	0.63	0.007	
32	Nitchitsu Kogyo Kaihatsu K.K.	Konan Refinery	1.90	2.70	0.084	Copper ore
33	Tohoku Hiryo K.K.	Akita Factory	7.05	3.68	0.005	Chamber muds
34	Mitsubishi Kogyo K.K.	Osaruzawa Refinery	6.31	1.76	0.073	
35	Nitto Ryuso K.K.	Shimonoseki Factory	33.86	1.06	0.006	Chamber muds
36	Kureha Boseki K.K.	Nishiki Factory	12.32	3.24	0.053	Sulfuric acid plant

Table 2. (*continued.*)

No.	Company	Factory	Moisture (%)	Sp. gr.	Thallium content (%)	Remarks
41	Fujita Min. Ind. Co., Ltd.	Kosaka Factory	2.56	3.54	0.003	Smelting Furnace
42	Fujita Min. Ind. Co., Ltd.	Kosaka Factory	17.93	4.78	0.104	Converter Furnace
43	Yahagi Chem. Ind. Co., Ltd.	Nagoya Factory	3.75	3.48	0.019	
44	Niigata Sulfuric Acid Ind. Co.	Yokohama Factory	5.65	3.22	0.001	Cottrell dust
45	Nitto Chem. Ind. Co., Ltd.	Nakagawa Factory	4.96	3.85	0.033	Sulfuric acid plant (contact process)
46	Nakagawa Chem. Ind. Co., Ltd.	Nakagawa Factory	6.87	3.52	0.000	Sulfuric acid plant (lead chamber)
47	Toyo Koatsu Kogyo K.K.	Omuda Factory	2.10	1.74	0.002	Hot Cottrell dust
48	Toyo Koatsu Kogyo K.K.	Omuda Factory	4.82	4.41	0.064	Mist Cottrell dust
49	Mitsubishi Min. Ind. Co., Ltd.	Hosonokura Refinery	3.04	1.33	0.039	Cottrell dust (lead)
52	Tagi Seihijo Co., Ltd.	——	17.44	1.33	0.040	Sulfuric acid plant
53	Tagi Seihijo Co., Ltd.	——	21.69	2.92	0.046	Sulfuric acid plant
60	Nitchitsu Kogyo Kaihatsu K.K.	Konan Refinery	1.19	1.38	0.033	Cottrell dust (lead)
61	Mitsubishi Min. Ind. Co., Ltd.	Hosonokura Refinery	6.24	3.66	0.070	Zinc ore

Conclusion. The spectroscopic detection of minute quantities of thallium in the flue-dust and chamber muds was frequently undertaken by several workers but its chemical determination was rare. And in the latter case, the thallium content determined chemically had been exceedingly large,[5] a thing which seldom occurs. For instance in one of the results a value as large as 6% had been reported. Such minute quantities of thallium as determined chemically by the author is impossible to be determined with any other method used hitherto.

Reasons for the superiority of the author's method responsible for the good result are attributed to the following: (1) Preliminary separation is unnecessary upon treatment with sulfuric acid and sulfur dioxide: 2) Moreover the complete and instantaneous precipitation of a minute quantity of thallium as $Co(NH_3)_6(Tl\,Cl_6)$ is made possible by the addition of ammonium chloride. Furthermore, this method is most remarkably accurate owing to its small factor of thallium as compared with any

(5) R. Böttger, See foot-note (1); T. Carstanjen, See foot-note (1); T Carstanjen and Marquart, *Verh. Naturhist. Vereins. Reuss. Rheinlande Westfalens*, **24** (1867), *Correspondenzblatt* S **102**; D. Playfair, *Chem. N.*, **39** (1879), 245; A. V. Petar, See foot-note (1).

other factor used hitherto. For example, it is smaller than a half of the factor in the chromate method (0.77895) which had been hitherto the most widely used. Data concerning the locality of these samples and the occurrence of the ores used in these factories were destroyed during the war. Every effort was made since to obtain as full and as complete information as possible regarding these matters but with little success. Though it may not be perfect, an attempt is being made to revive the result obtained from the data.

(1) Thallium Content: The greater part of the samples analysed contained less than 0.05% for each, the total amounting to approximately 67%. Only three samples No. 7, No. 12, No. 13, are extraordinarily rich in thallium, but such a large amount as has been reported by former workers can not be found. No. 12 and No. 13 were chamber muds from pyrites burnt in a sulfuric acid factory. But the nature of the pyrites is not definite owing to data having been lost. The flue-dust used for sample No. 7 was sent from Saganoseki Refinery, one of the largest and oldest refineries in Japan, producing zinc and lead.

(2) Relation between thallium content and specific gravity: There are no samples whose specific gravity lies between 2.1 and 2.9. Therefore these samples can be divided into the two following groups according to specific gravity: Group A, less than 2.1 and Group B, larger than 3.0. The former occurs mostly in flue-dust and the latter, strictly in chamber muds from the sulfuric acid factory. The total content of thallium in almost all the samples, 62% of the whole, is each less than 0.05%. Samples with thallium content larger than 0.05 are as follows: Group A—No. 2, No. 7, No. 20, No. 29, No. 34; Group—B No. 1, No. 4, No. 11, No. 12, No. 13, No. 18, No. 21, No. 32, No. 36, No. 40, No. 42, No. 45, No. 48, No. 61.

Among these groups A and B, No. 7, No. 12, and No. 13 are remarkably abundant in thallium.

(3) Color and Thallium Content: A distinct relation between color and thallium content cannot be observed. However the color of a sample belonging to Group B is dull brown, sepia, chocolate tinged, while on the other hand Group A is dark olive, smoke grey tinged. Carstenjen had reported that red-tinged samples are richer in thallium than grey-tinged samples. Similar conditions may be said of this case also.

The author wishes to express his sincere appreciation to Prof. K. Kimura for his suggestion of the problem and his continued supervision through the investigation. He is also indebted to Miss F. Ueda who has kindly undertaken to perform a part of the experiment.

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Inorganic and Analytical Chemistry Using Ion-Exchange Resin. I.

Vanadium Ion Exchange in Cation-Exchange Resin Column.

By Hidetake KAKIHANA.

(Received May 30, 1949.)

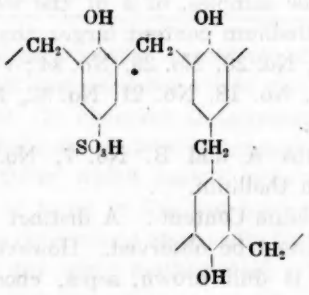
Introduction. How do amphoteric electrolytes react in the cation-exchange resin column? Though it has not yet been explained, it must be one of the most interesting problems in the chemistry using ion-exchange resin because of the possibility of its wide applications to the inorganic and analytical chemistry.(1)

In this report, the author has taken quinquivalent vanadium ion as the representative of amphoteric electrolytes, and has explained the behaviors of the ion in cation-exchange resin column.

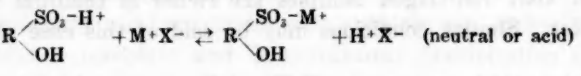
Property and Use of Cation-Exchange Resin.

(1) Type. The resin used in this research is phenol sulfonic acid type cation-exchange resin.

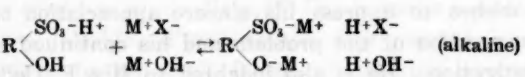
(2) Structure:



(3) The Formulas of Exchange Reactions:



(neutral or acid)



(alkaline)

(4) The Exchange Capacity. The exchange capacity of the resin used in this research is 1.74 milliequivalents per gram.

(1) It would be understood in the following papers.

(5) The Structure of the Resin Column.[2] A burette (B) is cut about 20 cm. long, in the bottom of which well-washed glass-wool is packed (0.5 cm. in thickness). Upon the glass-wool the cation-exchange resin (20 meches) is poured together with distilled water. (The apparent volume of resin layer is 10 c.c. in water). (The existence of bubbles in it should be avoided.) By means of a thin rubber-tube (C), the bottom of the burette is connected with a thin glass-tube (D) as shown in Fig. 1. The other end of the glass-tube is placed 0.5 cm. higher than the top of the resin layer in order to keep the solution level above the resin. Under it a beaker (E) is placed to collect the effluent. Upon the burette (B) a separating funnel (A) is placed so that the sample solution and washings may drop on the resin column.

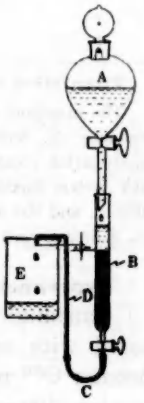


Fig. 1.

With such an apparatus, when the velocity of dropping is properly adjusted, the solution passes through the resin column at a constant rate.

(6) Method for Regeneration of the Resin. Add 1 N to 3 N hydrochloric acid to the resin in a beaker, then, after le ving them about 1 hour under occasional stirring, decant out the acid, wash with distilled water, and repeat these processes several times, thus the resin is almost perfectly washed. The resin thus obtained is put into the apparatus with distilled water, and washed perfectly by passing 500 c.c. of the acid and 500 c.c. of distilled water through it. In some cases, it is permitted to wash directly in the apparatus without treating it previously in the beaker.

(7) Method for Saturating the Sulfonic Group with H^+. The sulfonic group of the resin treated as suggested in (6) is completely saturated with H^+, and of course phenolic-OH group also remains in the form of OH.

(8) Method for Saturating Only the Sulfonic Group with Na^+ or NH_4^+. Wash the resin in a beaker as mentioned in (6), pour 1 N sodium chloride solution (pH, if necessary, is moderated about 7 with sodium hydroxide), or 1 N ammonium chloride solution (pH, also about 7 with ammonium hydroxide) into the beaker, and stir the resin thoroughly. After decantation, wash the resin twice with distilled water, add again 1 N sodium chloride solution (pH, about 7) or ammonium chloride solution (pH, about 7), and leave it for hours under occasional stirring.

Then, transfer the resin to the apparatus, pass 300–500 c.c. of 1 N sodium chloride or 1 N ammonium chloride solution (pH, about 7) through it, and then wash it with 500 c.c. of distilled water.

(9) Method for Saturating Both Sulfonic Group and Phenolic-OH Group with Na^+ or NH_4^+. By using alkaline solution of 1 N sodium chloride (pH, 10 ~ 12, with sodium hydroxide) or alkaline solution of 1 N ammonium chloride (pH, 10 ~ 12 with ammonium hydroxide), not only in the sulfonic group but also in the phenolic-OH group H^+ is exchanged with Na^+ or NH_4^+.

Therefore, if above-mentioned alkaline solution of 1 N sodium chloride or 1 N ammonium chloride is passed through the resin column till the pH of effluent becomes equal to that of the poured solution, not only sulfonic group but also phenolic-OH group of the resin is saturated with Na^+ or NH_4^+.

(10) Notations of the Resin Column. For convenience' sake, the resin columns are described by the following notations:

Cation-Exchange Resin (Phenol-sulfonic acid type):	C
Cation-Exchange Resin, when all groups remain H^+:	C^{HH}
Cation-Exchange Resin, when only sulfonic group is saturated with NH_4^+:	C^{NH_4H}

(2) Similar apparatus had been used by K. Helrich and W. Rieman III. (*Anal. Chem.*, **19** (1947), 651).

Cation-Exchange Resin, when only sulfonic group is saturated with Na^+ : C^{NaH}
Cation-Exchange Resin, when all groups are saturated with NH_4^+ : $C^{NH_4^+ NH_4^+}$
Cation-Exchange Resin, when all groups are saturated with Na^+ : C^{NaNa}

Preparation of Vanadium Standard Solutions.

The standard solution of vanadium (600γ V/c.c.) was prepared with ammonium metavanadate (E. Merck). It was standardized with normal solution of potassium permanganate after reducing it with sulfur dioxide (the excess of sulfur dioxide was expelled with carbon dioxide). Thus, 600γ of V/c.c. was decided. Then solutions containing 60γ/c.c., 6.0γ/c.c. and 0.6γ/c.c. were prepared. Similarly, the solutions of 261γ/c.c., 26.1γ/c.c., 13.1γ/c.c., 6.5γ/c.c. and 3.3γ/c.c. were also prepared.

Experiment I. Quinquivalent Vanadium Ion Exchange in C^{HH}.

Vanadium solutions of various concentrations and *p*H (*p*H was adjusted with ammonium hydroxide and hydrochloric acid) were passed through C^{HH} in this experiment. In each case, after passing the sample solution, 100 c.c. of distilled water was passed for washing (flow rate was 1.5 c.c./min.[3] and nearly the same in all the following experiments), effluent was gathered in a beaker, alkalized with ammonium hydroxide, concentrated to proper volume by heating on a water-bath, and neutralized with 0.2 N sulfuric acid using methyl orange as indicator after transferring the solution into a separating funnel. Then, vanadium in it was colorimetrically determined by the oxine-chloroform method[4] using the Pulfrich Stufen Photometer (Zeiss). The results are shown in Table 1.

Table 1.

Experiment number		1	2	3	4	5	6
Sample solution	*p*H	1.0	5.0	7<	7<	7<	12.0
	Amount of V γ/c.c.	0.6	1.2	1.2	6.0	6.0	0.6
	Amount of V Total amount (γ)	60.0	60.0	60.0	60.0	60.0	60.0
	Total volume (c.c.)	100.0	50.0	50.0	10.0	10.0	100.0
Amount of washings (c.c.)		100	100	100	100	100	100
V, found in effluent (γ)		0	0	0	0	0	0

As obviously shown in Table 1, quinquivalent vanadium is completely captured by C^{HH} independently of *p*H of the solutions. Therefore, C^{HH} cannot be used for the separation of vanadium from other cations.

(3) Accordingly, space velocity is 9.

(4) At a *p*H corresponding to the intermediate color of methyl orange, quinquivalent vanadium reacts with 8-hydroxyquinoline to give a compound soluble in chloroform with a Bordeaux red to black color. This sensitive and persistent color of the vanadium-8-hydroxyquinoline salt in chloroform layer can be used for the most sensitive and accurate colorimetric determination of this element.

Experiment II. Qninquivalent Vanadium Ion Exchange in C^{NaNa}.

Using C^{NaNa}, nearly the same process[5] as in Expt. 1 was carried out. The results are shown in Table 2.

Table 2.

Experiment number		7	8	9	10	11	12
Sample solution	*p*H	12.4	12.1	5.4	5.4	5.0	1.3
	Amount of V (*γ*/c.c.)	6.0	6.0	1.0	6.0	6.0	1.0
	Amount of V (Total amount (*γ*))	60.0	60.0	60.0	60.0	60.0	60.0
	Total volume (c.c.)	10.0	10.0	60.9	10.0	10.0	60.0
Amount of washings (c.c.)		50.0	50.0	50.0	50.0	50.0	50.0
V, found in effluent (*γ*)		59.6	59.4	60.1	60.0	60.2	17.5
Error	*γ*	−0.4	−0.6	+0.1	0.0	+0.2	−42.5
	%	−0.6	−1.0	+0.2	0.0	+0.3	−70.8

In Expts. 7, 8, 9, 10 and 11, where *p*H 5.0 or more is used, vanadium passes through C^{NaNa} within experimental error, while in Expt. 12, namely in case of *p*H 3, only a little part of vanadium is detected in the effluent.

Experiment III. Quinquivalent Vanadium Ion Exchange in C^{NaH}.

Using C^{NaH}, much the same process[6] as in Expt. 1 was carried out. The results are shown in Table 3.

Table 3.

Experiment number		13	14	15	16	17	18	19	20	21
Sample solution	*p*H	10.0	8.0	5.4	5.0	3.0	3.0	2.2	2.2	1.8
	Amount of V (*γ*/c.c.)	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
	Amount of V (Total amount (*γ*))	36.0	48.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
	Total volume (c.c.)	15.0	20.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Amount of washings (c.c.)		50.0	50.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
V, found in effluent (*γ*)		36.4	−8.5	24.0	24.0	21.3	20.4	16.9	16.5	7.0
Error	*γ*	−0.4	−0.5	0.0	0.0	− 2.7	− 3.6	− 7.1	− 7.5	−17.0
	%	−1.1	−1.0	0.0	0.0	−11.3	−15.0	−29.6	−31.3	−70.9
$\frac{\text{V found in effluent}}{\text{V in sample solution}} \times 100$		101.1	101.0	100.0	100.0	88.7	85.0	70.4	68.7	29.1

In Expts. 13, 14, 15 and 16, namely where *p*H 5.0 or more is used, vanadium passes through C^{NaH} within the experimental error. And, in

(5) Ammonium hydroxide was not added before concentrating the effluent.

(6) The *p*H of sample solutions were adjusted with ammonium hydroxide and nitric acid. Ammonium hydroxide was not added before concentrating the effluent.

Expts. 17–21, namely *p*H 3 (Expts. 17 and 18), *p*H 2.2 (Expts. 19 and 20) and *p*H 1.8 (Expt. 21), the lower the *p*H of the sample solutions, the larger the amount of vanadium captured by C^{NaH} becomes.

Experiment IV. Quinquivalent Vanadium Ion Exchange in C^{NH_4H}.

In Expts. I, II and III, the outline of the quinquivalent vanadium ion exchange in the column of cation-exchange resin is shown, so, in the next experiments the more detailed study has been carried out through C^{NH_4H}, which is very convenient for various practical and theoretical purposes.

Table 4.

Experiment number		22	23	24	25	26	27	28	29	30
Sample solution	*p*H	7.6	7.2	6.4	6.4	5.0	5.0	4.7	4.2	3.8
	Amount of V: γ/c.c.	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
	Amount of V: Total amount (γ)	26.1	52.2	13.1	13.1	13.1	26.1	26.1	26.1	26.1
	Total volume (c.c.)	10.0	20.0	5.0	5.0	5.0	10.0	10.0	10.0	10.0
Amount of washings (c.c.)		60.0	60.0	30.0	30.0	30.0	60.0	60.0	60.0	60.0
ε of effluent		0.466	0.905	0.264	0.258	0.260	0.412	0.460	0.405	0.169
V, found in effluent (γ)		26.0	51.9	13.2	13.0	13.1	26.2	25.6	22.1	8.0
ε_0 (Theoretical value)		0.469	0.911	0.261	0.261	0.261	0.469	0.461	0.469	0.160
Error	$\varepsilon - \varepsilon_0$	−0.003	−0.006	−0.003	−0.003	−0.001	−0.003	−0.009	−0.064	−0.300
	γ	−0.1	−0.3	−0.1	−0.1	0	−0.1	−0.5	−4.0	−18.1
	%	−0.4	−0.6	−0.8	−0.8	0	−0.4	−1.9	−15.3	−69.3
$\frac{\text{V found in effluent}}{\text{V in sample solution}} \times 100$		99.6	99.4	100.8	99.2	100.0	100.4	98.1	84.7	30.7

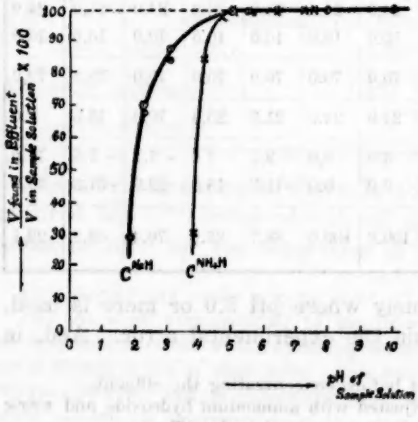


Fig. 2.

After passing vanadium solution (in this case *p*H was adjusted with ammonium hydroxide and sulfuric acid) through C^{NH_4H} and washing with 30–60 c.c. of distilled water, effluent was gathered in a separating funnel and neutralized with 0.2 N sulfuric acid using methyl orange as indicator. Then, vanadium in it was colorimetrically determined by the oxine-chloroform method. The results are shown in Table 4.

In Expts. 22, 23, 24, 25, 26 and 27, namely, where *p*H 5.0 o

more is used, vanadium exactly passes through C^{NH_4H}. However, as *p*H decreases, the amount of vanadium captured by C^{NH_4H} increases just as in Expt. III.

In Fig. 2, the relation between the vanadium found in effluents and *p*H of sample solutions is illustrated in the case of C^{NaH} and C^{NH_4H}.

Conclusion.

Quinquivalent vanadium in aqueous solution is thoroughly captured by C^{RH} independently of *p*H of passing solutions. It passes, however, quantitatively through C^{NaNa}, C^{NaH} or C^{NH_4H}, if the *p*H of the passing solution is higher than 5.0; but it is partially captured by them, when the *p*H is lower than 5.0.[7]

The author wishes to thank Dr. Eiiti Minami, Professor of Analytical Chemistry, Tokyo University, and Mr. Masatake Honda, who have given him kind advice and suggestion in this problem.

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Tokyo University.

The Sunflavor of Beer. II. Essential Substance of Sunflavor.

By Yataro OBATA and Tei YAMANISHI.

(Received May 30, 1949.)

As reported in the previous paper[1] we have ascertained that the essential substance of sunflavor of beer is thioglycolic acid ethylester, and we could also prove chemically and biologically that the essential substance was produced from dithiodiglycolic acid diethylester (-S-S-) which originated in cystine within malt juice by the reducing function of certain unknown components in beer under exposure to sunlight.

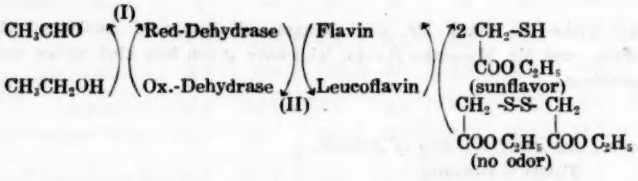
It has already been recognized that the malt of the raw material of beer contains the ferments belonging to dehydrase[2] and flavine[3] and that the solution of autolysed beer-yeast also contains a large amount of dehydrase[4] and flavin[5]. So we can easily expect that at least these

(1) Y. Obata and T. Yamanishi, *Agriculture*, (Japan) **9** (1947), 537.
(2) A. Foder and L. Frankenthal, *Biochem. Z.*, **225** (1930), 417.
(3) P. Karer and K. Schöpp, *Helv. Chem. Acta.*, **17** (1934), 1013.
(4) H. v. Euler and H. Adler, *Z. prakt. Chem.*, **235** (1935), 122.
(5) O. Warburg and W. Christian, *Biochem. Z.*, **266** (1933), 377.

ferments in beer yeast will be excreted into beer while its fermentation proceeds.

In fact it is reported that beer contains about 0.26 mg. of flavin per liter[6], and we could prove the existence of dehydrase in un-Pasteurized beer by the fact that such beer discolored methylene blue solution.

As we could presume that the flavin and dehydrase would take part in the development of the sunflavor of beer, we carried out an experimental study on the mechanism of sunflavor production with dehydrase prepared from beer yeast as well as with flavin. The result was just as we had presumed. Namely, we could prove that by the joint function of these two factors -S-S- was reduced and consequently sun-flavor was produced. This mechanism can be shown with chemical formula as follows:



The change of flavin to leucoflavin by dehydrase is accelerated by sunbeam.

Experimental Part.

Preparation of dehydrase solution. (i) Preparation of dehydrase acetone-sample. Concentrated suspension of beer-yeast which was previously washed twice with water, is cooled with ice, to which is added cold acetone gradually while stirring vigorously. By this treatment the cell membranes of beer-yeast are broken, and the suspension becomes voluminous.

Then it is rapidly filtered by suction, washed twice with acetone and finally washed once more with ether. By drying the residue in vacuum desiccator for one day white powder is obtained.

(ii) Preparation of dehydrase solution. 5 g. of dehydrase-acetone sample thus prepared is suspended in 100 c.c. of K_3PO_4 solution (*p*H 7.2) and kept in the thermostat at 28°C for two hours. When it is filtered, a clear pale yellow colored solution is obtained.

Experiment on the sunflavor production. (1) By dissolving K_3PO_4 in 3% alcohol, the latter is made to have *p*H 7.2 (optimum *p*H of alcohol-dehydrase). Two colorless glass vessels with a glass-stopper containing 10 c.c. are filed with the solution composed of 8 c.c. of the above mentioned alcohol, 2 c.c. of dehydrase solution and 1 mg. of flavin (Sankyo's injection drug).

(6) S. Komatsu et. al., *Chem. and Eng.*, (Japan) **41** (1938), 158.

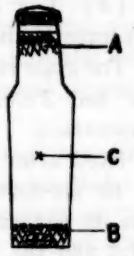
One bottle is kept in darkness and the other is exposed to sunlight.

After 90 min., the content of the former shows no change, while that of the latter becomes completely colorless and tells the change of flavin to leuco-flavin.

If one drop of -S-S- is sunk in this colorless liquid, the yellowish color comes back around -S-S-.

This means flavin is changed to the oxidized form. Accompanied with this change sunflavor is clearly produced.

If the solution used in this experiment lacks 3% alcohol or keeps its acidity at *p*H 5.4, its discoloration after 90 min. exposure to sunlight will be very faint.



A: Yellow, oxidation by air.
B: Yellow, oxidation by -S-S-.
C: Colorless.

Thus it can be concluded that in the reaction-system under consideration alcohol is necessary as its substrate and the optimum *p*H is at 7.2. These conditions coincide with those concluded in the previous report of one study connected with the sunflavor production by beer-yeast.

(2) On examining if dehydrase or flavin added separately can generate the sunflavor, it was concluded that their joint addition was necessary to induce the phenomenon in question as shown in Table 1.

Table 1.

Condition	Reaction system and sunflavor production	
Exposed in sunlight	-S-S- (−)	-S-S-+D (±)
,,	-S-S-+F (±)	-S-S-+D+F(*) (+)
Kept in darkness	-S-S-+F (−)	-S-S-+D+F (−)

(+) and (−) denote respectively that sunflavor is generated or not generated after 90 min. -S-S- denotes the substrate solution of the reaction which is at *p*H 7.2 being composed of 100 c.c. of the saturated solution of dithiodiglycolic acid diethylester, 3 c.c. of alcohol and 2.5 gr. of K_3PO_4. F denotes 2 mg. flavin (injection drug) dissolved in 20 c.c. of K_3PO_4 solution (*p*H 7.2). D denotes dehydrase solution prepared from beer yeast as in the experiment (1).

These three kinds of solution are mixed in the following proportion. One c.c. each of D and F are taken and poured together in the glass vessel explained in the experiment (1).

Then the remaining space of the vessel is filled up with -S-S-.

As the vessel is thus filled up with reaction solution perfectly, the experiment is carried out under an anaerobic condition.

(3) Test on the presence of dehydrase in beer and destruction of dehydrase by heating.

The glass vessel explained above was filled with 8 c.c. of un-Pasteurized beer and 2 c.c. of $^1/_{25000}$ methylene blue solution, and kept at room temperature.

Even after 3 days the color of the solution showed little change.

To accelerate the reaction *p*H of un-Pasteurized beer was raised from its natural value 4.2 to the optimum for the function of dehydrase or 7.2 and the vessel was placed in the thermostat regulated at 22°C. The color of the solution then faded perfectly after 2 days.

Un-Pasteurized beer comes very quickly to give off sunflavor while Pasteurized beer does so very slowly. This fact suggests that the dehydrase becomes functionless by heating. Pasteurized beer, which had previously been heated at 60°C for 30 min., was examined by the methylene blue test under the same condition as the preceding experiment. The solution was unchanged in color even after 3 days.

This proves the perfect loss of the dehydrase function in Pasteurized beer.

On the other hand, if the reaction-system (*) in the experiment (2) had previous been heated at 60°C for 30 min., methylene blue was not reduced and sunflavor was not given off by exposing the solution to sunlight.

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Inhibitory Effect of Cyanine- and Styryl-Dyes upon Cholinesterase. I.

By Eiji HASE, Yoshihisa MIZUNO and Masujiro KATAYANAGI.

(Received June 13, 1949.)

During the past several years it has repeatedly been reported that certain cyanine- and styryl-dyes, usually known as photosensitizers,[1] have some therapeutic effect against certain kind of diseases.[2] The mechanism

(1) T. Ogata, "Photosensitive Dyes" ("Kankō-shikiso" in Japanese), Sankaidō-shoten, Tokyo, 1941.

(2) A. D. Welch, L. Peters, E. Beuding, A. Valk and A. Higashi, *Science*, **105** (1947), 486; L. G. S. Brooker and L. A. Swett, *Science*, **105** (1947), 496.

of this chemotherapeutic action remains as yet wholly unknown. Not only from the pharmacological point of view, but also from the purely enzymological view-point, it may be worth while to investigate the action of these substances which have the peculiar physico-chemical properties upon the activities of different enzymes.

Brooker and Swett[2] have reported that cyanine- and styryl-dyes have no appreciable effect upon various oxidative enzymes studied. On the other hand, Hino and his coworkers[3] have shown that some of these dyes exhibit strong inhibiting action upon catalase. In the present study, it is shown that certain substances belonging to the same category exert remarkable suppressing effect upon the activity of cholinesterase. Whether this effect has anything to do with the chemotherapeutic action reported or not is the problem to be worked out in the future.

Experimental Method.

The activity of cholinesterase was measured by the potentiometric titration method after Alles and Hawes[4] with a slight modification for our experimental purpose. The dyes were dissolved in one per cent ethyl alcohol solution.* The mixture of enzyme and dye was regulated to *p*H 7.5 and left in a thermostat at 30°C for 10 min. After the addition of the substrate, the acid liberated by hydrolysis of ester was constantly neutralized with 0.04 N NaOH and its volume consumed was recorded against time. The estimation of the reaction rate was made on the initial part of the alkali-time curve thus obtained. The concentration of the substrate used was sufficiently high so that the rate of hydrolysis remained practically constant during the course of the experiment (10 min.).

Results.

Inhibition of the cholinesterase activity in horse serum by certain cyanine- and styryl-dyes. The inhibitory effects of about one hundred of cyanine- and styryl-dyes were examined. It was found that almost all of them show more or less strong inhibitory action upon cholinesterase activity in horse serum. Several examples of these dyes which exhibit most strong inhibitory effects are shown in Table 1. Their efficacies are compared in terms of concentration causing 50 per cent inhibition with those of substances which have thus far been known as strong inhibitors of cholinesterase.

It may be seen that the action of dyes is by far stronger than most of the substance listed, although it is less than one hundredth of the activity of eserine.

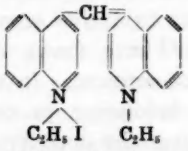
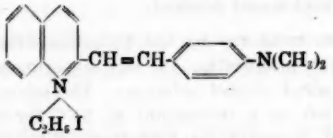
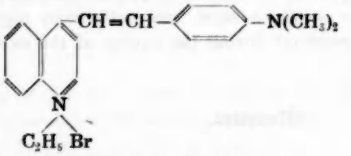
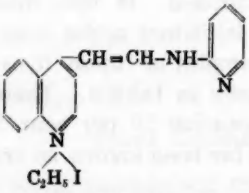
(3) S. Hino, Y. Ogura, M. Fujita and K. Shibata, Unpublished paper read before the Chem. Soc. Jap. in 1948.

(4) G. A. Alles and R. C. Hawes, *J. Biol. Chem.*, **133** (1940), 377.

* It was ascertained that ethyl alcohol had no effect upon cholinesterase under these experimental conditions.

Table 1.

Inhibitory effects of some cyanine- and styryl-dyes upon cholinesterase in horse serum.

Inhibitor		Concentration of the inhibitor causing 50% inhibition (mol/l.).
Cyanine-dyes	 1, 1′-Diethyl-4, 4′-monomethine-quinocyanine iodide	$10^{-5.4}$
	$C_{20}H_{19}S_2N_2I$	$10^{-5.5}$
Styryl-dyes	 *p*-Dimethylamino-benzyliden-quinaldine ethiodide	$10^{-5.6}$
	 *p*-Dimethylamino-benzyliden-lepidine ethobromide	$10^{-5.4}$
	 4-(α-Pyridylamino)-ethenyl-quinoline ethiodide	$10^{-5.4}$
Other substances	Eserine salicylate	$10^{-7.9}$
	Strychnine sulphate	$10^{-4.6}$
	Quinine hydrochloride	$10^{-4.1}$
	Atropine sulphate	$10^{-2.6}$

All these measurements were carried out under the same experimental conditions; *p*H of the reaction mixture, 7.5; concentration of the substrate (acetylcholine chloride), 0.02 mol/l.; the temperature, 30°C.

Effects upon the "specific" and "non-specific" cholinesterase. Recently it has been found[5] that there are two types of cholinesterase, the "specific" and the "non-specific". Using one of the most active cyanine dyes found in the previous experiment, the susceptibility of the two different types of cholinesterase was compared. As may be seen in Table 2 the non-specific cholinesterase seems to be more strongly inhibited than the specific one. Since, however, the preparation of the specific cholinesterase used in this experiment was not sufficiently homogeneous, the results obtained do not allow us to conclude definitely and quantitatively the difference in the susceptibilities of the two cholinesterases.

Table 2.

Effects of some cyanine- and styryl-dyes upon various esterases.

Dye	Enzyme	Source of enzyme	Substrate	Concentration of the dye causing 50% inhibition of enzyme activity (mol/l.)
1, 1′-Diethyl-4, 4′-monomethine-quinocyanine iodide	Non-specific cholinesterase	Horse serum	Acetylcholine chloride (0.02 mol/l.)*; *p*H, 7.5; 30°C	$10^{-5.4}$
,,	Specific cholinesterase	Homogenate of horse brain	Acetylcholine chloride (0.002 mol/l.)*; *p*H, 7.5; 30°C	$10^{-4.6}$~$10^{-4.8}$
,,	Esterase	Horse serum	Methylbutyrate and triacetin at various concentrations; *p*H, 7.5; 30°C	Slight activation
Various cyanine- and styryl-dyes	Esterase	Pig liver extract	Methylbutyrate (0.1 mol/l.); *p*H, 7.5; 30°C	No measurable effect

* Concentration of substrate applied was nearly saturating for the reaction velocity observed in each case.

Effects upon other esterases. Effects of cyanine- and styryl-dyes upon the esterase in horse serum were examined, methyl butyrate and triacetin being used as the substrate. It was found that some of the dyes examined increase slightly the activity of this enzyme and others have no measurable effects. It was further ascertained that the dyes have no appreciable effects upon the activity of liver esterase (Table 2).

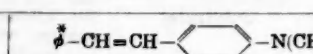
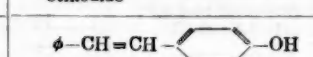
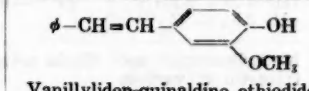
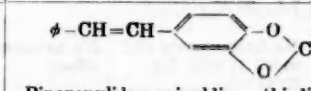
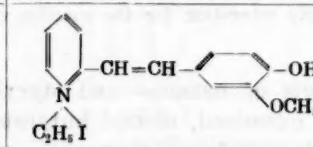
At any rate, it can be concluded that the inhibitory effect of the dyes is quite specific to the cholinesterase, whether "specific" or "non-specific".

(5) A. M. Wynne, *Ann. Rev. Biochem.*, **15** (1946), 63.

Relationship between the chemical constitution and the inhibitory effect. Comparison of efficacy of structurally related styryl-dyes shows us that there exists certain relationship between structure and inhibitory effect (see Table 3). It can be seen from the table that the dimethylamino radical, attached to benzene ring in the molecule, has the most favorable effect upon the inhibitory action of the dye.

It is another interesting problem that vanillylidenpicolin ethiodide, which contains a pyridine nucleus, has no appreciable effect upon cholinesterase, while vanillylidenquinaldine ethiodide, possessing a quinoline nucleus, exhibits a considerable inhibitory action under the same experimental conditions.

Table 3. Relationship between the chemical constitution of the related styryl-dyes and their inhibitory effects upon the cholinesterase in horse serum.

	Chemical structure of dye	Concentration of the dye causing 50% inhibition (mol/l.)**
I	 *p*-Dimethylamino-benzyliden-quinaldine ethiodide	$10^{-5.6}$
II	 *p*-Hydroxybenzyliden-quinaldine ethiodide	$10^{-5.0}$
III	 Vanillyliden-quinaldine ethiodide	$10^{-4.8}$
IV	 Piperonyliden-quinaldine ethiodide	$10^{-4.7}$
V	 Vanillyliden-picoline ethiodide	No appreciable effect even at the concentration of $10^{-4.0}$ mol/l.

* The symbol ϕ represents the quinaldine ethiodide nucleus

** Experimental condition; *p*H, 7.5; temperature, 30°C; concentration of substrate (acetylcholine chloride), 0.02 mol/l.

Summary.

(1) It was found that a number of cyanine- and styryl-dyes has a strong inhibitory action upon cholinesterase.

(2) Both the specific and non-specific cholinesterase are inhibited by the subtances, the latter being probably more susceptible than the former.

(3) The substances have no appreciable effects upon the liver esterase. With the esterase in horse serum, even a slight acceleration of the activity was observed under the influence of some dyes investigated.

(4) For some related members of styryl-dyes, a certain relationship between chemical constitution and their inhibitory effects upon cholinesterase could be found.

We wish to express our sincere gratitude to Professor H. Tamiya and Doctor A. Takamiya for their kind guidance and to Mr. Innami for his assistance in this work. These experiments were largely supported by the Scientific Research Encouragement Grant and the Grant-in-aid for Private Research Institute donated by the Ministry of Education, to which our thanks are due.

The Tokugawa Institute for Biological Research.

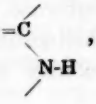
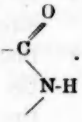
On the Configurations of the Peptide Linkage in Proteins and Some Other Substances, as Revealed by Their Near-Infrared Absorption Spectra.

By **Masamichi TSUBOI.**

(Received June 29, 1949.)

As continuation of his investigation on the hydrogen bondings in compounds with the peptide linkage,[1] the writer made 3μ region infrared absorption measurements of some proteins, diketopiperazine, some amides, some lactams, etc., in the solid or the liquid state. In this paper the writer reports some results of these measurements and gives interpretations of them in the light of the knowledge, previously obtained,

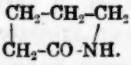
(1) M. Tsuboi, This Bulletin, **22** (1949), 215.

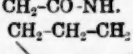
of the 3μ region absorptions of amides in carbon tetrachloride solutions.[1] A conclusion reached is that the peptide linkage in proteins is of the *trans* configuration,  , but not of the *cis* configuration,  .

Experimental.

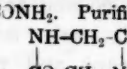
The infrared monochromator and other apparatus used in the present investigation are the same as those described in the previous paper.[1] The samples examined are:

N-Methylacetamide, $CH_3CONHCH_3$. Purified by vacuum distillation. Melting point: 28°C.

δ-Valerolactam,  . Purified by vacuum distillation. Melting point: 40°C.

ε-Caparolactam,  . Supplied by K. Hoshino and H. Yumoto. Purified by vacuum distillation. Melting point: 68°C.

Formamide, $HCONH_2$. Purified by vacuum distillation.

Diketopiperazine,  . Prepared by A. Mukai. Purified by repeated recrystallization from hydrochloric acid using methanol.

Poly-ε-capramide ("Amilan").[2] $\ldots(CH_2)_5CONH(CH_2)_5CONH\ldots$. Supplied by K. Hoshino and H. Yumoto. Dried over calcium chloride.

Egg albumin. Prepared according to the method of Kekwick, and purified by recrystallization by H. Sugano. A suitable film for the infrared absorption measurements was obtained by spreading a drop of aqueous solution of egg albumin on a cover glass (the salt contained being removed by dialysis), and drying it first over calcium chloride and then over phosphorus pentoxide.

Horse serum albumin. Prepared according to the method of Kekwick, and purified by recrystallization by S. Nakamura. A film of this protein was prepared in the same way as that of egg albumin.

Results and Interpretations.

The results obtained are shown in Fig. 1, where $\log I_0/I$ is plotted against wave-length, I_0/I being the ratio of the intensity of light transmitted by blank space to that by the film.

Before proceeding to the interpretation of these results, the assignments of N-H absorption bands of compounds with the peptide linkage, described in detail in the previous paper,[1] may be summarized in Table 1. These relations were obtained by studying the effects of the concentrations and the temperatures of carbon tetrachloride solutions of N-methylacetamide and several other amides and δ-valerolactam on the 3μ region absorption spectra. Compounds with the peptide linkage show the strong tendency of forming intermolecular and intramolecular N-H...O=C bondings; accordingly most of N-H's and C=O's in these compounds

(2) Material of synthetic fibers, made by Tōyō Rayon Co.

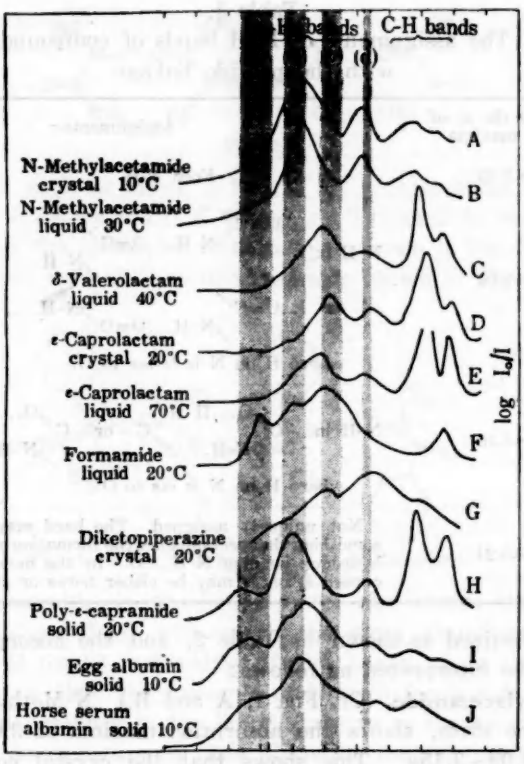
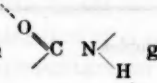
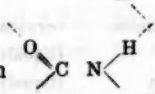
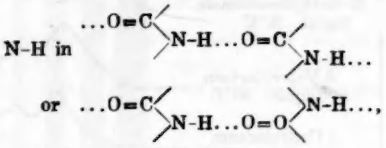
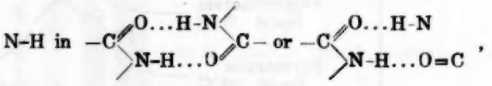


Fig. 1. 3μ region infrared absorption curves of some compounds with peptide linkage.

must form hydrogen bondings in the liquid and the solid states. On the other hand, as is shown in Table 1, N–H in  gives an association band in 2.97~3.04μ region, and N–H in  an association band in 3.09~3.15μ region.* Hence, *we can tell whether the peptide linkage is of the trans configuration or of the cis configuration, according as the N–H association band appears in the 2.97~3.04μ region or the 3.09~3.15μ region.* Using this relation, the configurations of CONH

* This difference in wave-lengths of N–H association bands is attributable to the difference in the positions of H relative to other atoms, as detailed in the author's previous paper.[1]

Table 1.
The assignments of N–H bands of compounds with the peptide linkage.

Wave-lengths (in μ) of absorption maxima	Assignments
(a) 2.83~2.93	Free N-H
(b) 2.97~3.04	N-H in  where H on N is *trans* to O.
(c) 3.09~3.15	N-H in  where H on N is *cis* to O.
(d) 3.22~3.24	Not uniquely assigned. The band may be assigned to something that accompanies the formation of intermolecular hydrogen bonding N-H...O. In the molecules here concerned H on N may be either *trans* or *cis* to O.

may be determined as shown in Table 2, and the absorption curves in Fig. 1 may be interpreted as follows:

N-Methylacetamide. (Cf. Fig. 1, A and B.) N-Methylacetamide, in the crystalline state, shows the absorption maxima at 3.03μ and 3.22μ, but not at 3.09~3.15μ. This shows that the crystal consists only of *trans* molecules (molecules in each of which H on N is *trans* to O), and

Table 2.
Configurations of CONH, determined from the wave-lengths of absorption maxima of N–H...O bands.

Samples		Wave-lengths (in μ) of absorption maxima of N–H...O bands			Configurations of CONH
N-Methylacetamide	(crystal)	3.03		3.22	trans
,,	(liquid)	3.03		3.22	trans
δ-Valerolactum	(liquid)		3.09	3.23	cis
ϵ-Caprolactam	(crystal)		3.11	3.24	cis
,,	(liquid)		3.09	3.23	cis
Formamide	(liquid)	3.00	3.10		trans and cis
Diketopiperazine	(crystal)		3.11	3.26	cis
Poly-ϵ capramide	(solid)	3.00		3.23	trans
Egg albumin	(solid)	3.04		3.24	trans
Horse serum albumin	(solid)	3.03		2.25	trans

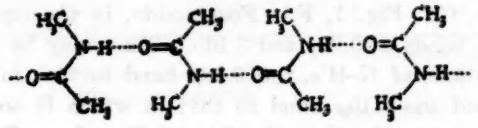


Fig. 2.

has a structure such as shown in Fig. 2. It is interesting to note that, according to C. J. Brown and D. E. Corbridge,[3] acetanilide has the crystal structure (hence the molecular structure) as shown in Fig. 3, which is of the same type as that of N-methylacetamide shown in Fig. 2.

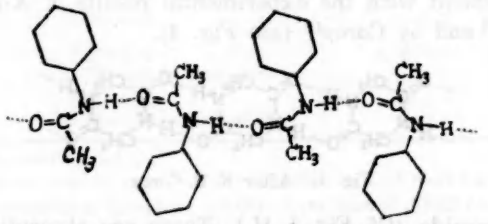


Fig. 3. After C. J. Brown and D. E. Corbridge.

N-Methylacetamide, in the liquid state, also shows the absorption maxima at 3.03μ and 3.22μ with nearly the same intensities (relative to those of the C–H bands) and widths of the bands as in the crystalline state. This shows that the molecule of N-methylacetamide is linked in nearly the same manner in the liquid state as in the crystalline state, at least at the temperatures a little above its melting point.

δ-Valerolactam. (Cf. Fig. 1, C.) δ-Valerolactam, in the liquid state, shows absorption maxima at 3.09μ and 3.23μ, i. e. at wave-lengths, respectively, shorter than 3.11μ and 3.24μ, which are the wave-lengths of absorption maxima of δ-valerolactam in carbon tetrachloride solution.[1] This fact may be explained as due to some structural deformation of the ring dimers (to which the 3.11μ and 3.24μ bands were assigned[1]), caused by closer mutual approach of the molecules of δ-valerolactam.

ε-Caprolactam. (Cf. Fig. 1, D and E.) ε-Caprolactam, in the crystalline state, shows absorption maxima at 3.11μ and 3.24μ, but not at 2.97~3.04μ. This shows that in ε-caprolactam molecules in the crystalline state, H on N is *cis* to O.

ε-Caprolactam, in the liquid state, shows two N–H association bands whose maxima lie at 3.09μ and at 3.23μ; hence, in this molecule H on N is *cis* to O, also in the liquid state, although these two bands are shorter in wave-lengths, weaker in absorption intensities (relative to those of the C–H bands), and broader than the 3.11μ and 3.24μ bands of ε-caprolactam in the crystalline state.

(3) C. J. Brown and D. E. Corbridge, *Nature*, **162** (1948), 72.

Formamide. (Cf. Fig. 1, F.) Formamide, in the liquid state, gives N–H association bands at 3.00μ and 3.10μ. They may be assigned to the stretching vibrations of N–H's, the 3.00μ band to that in which H on N is *trans* to O, and the 3.10μ band to that in which H on N is *cis* to O. Besides, it also gives a band at the free N–H region. This reveals that N–H's of formamide are partially in free state even in its pure liquid state—an unusual phenomenon for an associated liquid.

Diketopiperazine. (Cf. Fig. 1, G.) Diketpiperazine, in crystalline state, shows N–H bands at 3.11μ[4] and 3.26μ, but not at 2.97~3.04μ, revealing that the CONH of this substance is of the *cis* configuration. This is in agreement with the experimental results of X-ray analysis by Go and Ohashi[5] and by Corey[6] (see Fig. 4).

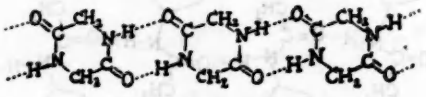
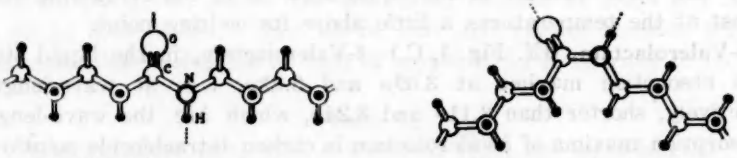


Fig. 4. After R. B. Corey.

Poly-ε-capramide. (Cf. Fig. 1, H.) There are absorption maxima for poly-ε-capramide at 3.00μ and at 3.23μ, but none in the 3.09~3.15μ region.[7] This shows that H on N in poly-ε-capramide is *trans* to O. It follows then that the chain ...$-CH_2CH_2CONHCH_2CH_2-$... in this substance must be of the form as shown in Fig. 5 (A), but not of the form as shown in Fig. 5 (B).



(A) H on N is trans to O. (B) H on N is cis to O.

Fig. 5.

Proteins. (Cf. Fig. 1, I and J.) The molecules of proteins have as their constituents various atomic groups that give absorption bands in the 3μ region. They are shown in Table 3, with the wave-lengths of

(4) The 3μ region absorption curve of solid film of diketopiperazine has already been obtained by A. M. Buswell and others in 1940 (*J. Am. Chem. Soc.*, **62** (1940), 2759). They observed an absorption at 3.14μ, but they suspected this to be due to glycine, which might be produced as a dissociation product in the preparation of the film. The present writer observed an absorption at 3.11μ (probably corresponding to that at 3.14μ observed by Buswell) even in a film confirmed to be free from glycine by the K. Kuratani's infrared absorption measurements in the rock salt region.

(5) Go and Ohashi, Paper communicated to Chem. Soc. Japan, at its 60th Annual Meeting, April 1938.

(6) R. B. Corey, *J. Am. Chem. Soc.*, **60** (1938), 1598.

(7) See foot-note (9).

Table 3.

Atomic groups	Wave-lengths (in μ) of absorption maxima	Number of the groups in one molecule	
		Egg albumin	Bovine serum albumin
$\rangle$C–H	3.35~3.60	2050	3390
$\rangle$C–H[a]	3.21~3.30	150	270
CONH	2.97~3.26 (2.83~2.93 in free state)	430	690
$\overset{+}{N}$–H	3.14~3.28	80	180
N–H[b]	2.83~2.95		
COOH[c]	3.27~3.89 (2.79~2.84 in free state)	< 50[c]	< 90[c]
OH	2.83~3.22 (2.75~2.77 in free state)	50	80

(a) C–H with unsaturated C.
(b) Amino group may be mostly of N^+–H form, that of N–H form being negligible.
(c) Carboxyl group may be mostly of COO^- form, that of COOH form being negligible.

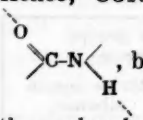
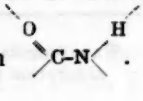
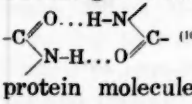
the absorption maxima and the number of each of the atomic groups in one molecule of egg albumin and of bovine serum albumin, estimated from the results of amino acid analyses of these proteins.[8] As shown in the table, COHN is the most abundant of the atomic groups that give absorption maxima in the 2.97~3.26μ region. It is this group that determines the essential forms of the absorption curves of these proteins in the said region. Other groups, such as N^+–H and O–H, being far less in number, their effects upon the forms of the curves must be very little. The group $\rangle$C–H, on the other hand, is comparatively large in number, attaining as many as one third of the number of CONH; but since $\rangle$C–H bands are generally much weaker than N–H association bands in absorption intensities (per one group), its effect is considered to be also negligible. The absorption maxima in the same region of egg albumin and horse serum albumin are at 3.04μ or 3.03μ and at 3.24μ or 3.25μ,[9] but not in the vicinity of 3.11μ, unlike δ-valerolactam, ε-caprolactam, and diketopiperazine, all of which show the absorption maximum near 3.11μ. Other proteins, according to Buswell and others,[10] also show the absorption maxima at nearly the same positions, except salmin.[11]

(8) Block, *Advances in Protein Chemistry*, **2** (1945), 119; Stein and Moore, *J. Biol. Chem.*, **178** (1949), 79.

(9) In the last three curves of Fig. 1, besides the absorption maxima mentioned above, there are weak ones at 2.85~2.88μ, which may probably be attributed to the bound water.

(10) A. M. Buswell, K. F. Krebs and W. H. Rodebush, *J. Phys. Chem.*, **44** (1940), 1126.

(11) The 3.09μ band of salmin may be due to the N–H's in guanidinium groups, as Buswell and others indicated in their paper cited in (10).

Hence, CONH in these proteins must be of the *trans* configuration  , but not of the *cis* configuration  . This is due to the molecular structure of proteins, which has been much debated. On this basis any structural view involving the assumption of the *cis* configuration in the peptide linkage is excluded, such as the view assuming the diketopiperazine structure, or the ring dimer structure,  [10], etc. On the other hand, the structural model of protein molecule recently proposed by Simanouti and Mizushima[12] is incidentally in accord with the writer's conclusion given above, for in their model the peptide linkage is always and exclusively of *trans* configuration.

The writer wishes to express his sincere thanks to Professor Sanichiro Mizushima and Assistant Professor Takehiko Simanouti for their kind guidance throughout this work.

Chemical Institute, Faculty of Science,
Tokyo University.

Studies on Sinomenine. LXI.
On (+)-epi-Dihydro-thebainone.

By Kakuji GOTO and Kimiyo MICHI.

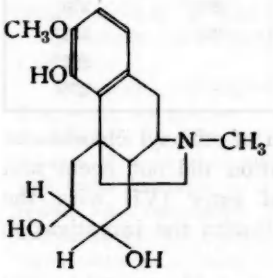
(Received July 6, 1949.)

In a previous communication,[1] we reported the formation of (+)-dihydro-codeinone (III) from (+)-dihydro-sinomeninone (II) by boiling the latter in 50% sulfuric acid solution. We applied the same treatment to tetrahydro-sinomeninone (I) to see whether the base is thereby led to the formation of the oxide ring or not. We found, however, that no trace of alkali-insoluble base was formed in this case, but tetrahydro-sinomeninone was transformed into a base (IV) isomeric with the well-known dihydro-

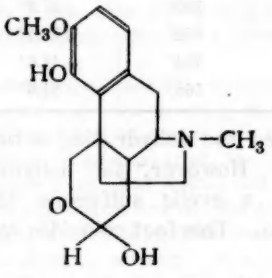
(12) T. Simanouti and S. Mizushima, *Kwagaku*, **17** (1947), 24, 52; This Bulletin, **21** (1948), 1.

(1) Part LX, *Acta phytochimica*, XV, 187 (1949).

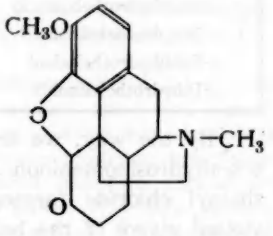
thebainone. The transformation is brought about undoubtedly by a kind of semi-pinacoline rearrangement.



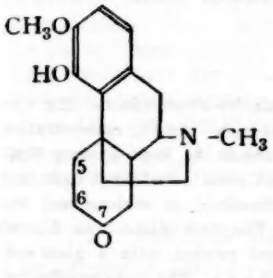
I



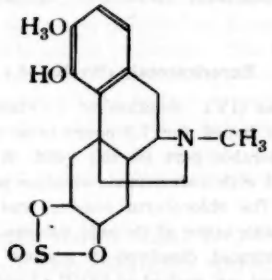
II



III



IV



VI

V (C_7 = CHOH)

The formation of (+)-dihydro-codeinone (III) from α-dihydro-sinomeninone (II) necessitates the migration of the hydroxyl groups from C (7) to C (5) around the ketonic group at C (6). This may be effected by anionotropy through the interchange of the positions between the hydroxyl group and the double linking, formed by the enolization of the ketone. The fact that tetrahydro-sinomeninone was not led to the oxide ring closure by the same treatment seems to verify the above supposition to some degree.

The new base, epi-dihydrothebainene, having the molecular formula $C_{18}H_{23}O_3N$ and being soluble in caustic alkali, gives a ferric chloride reaction and forms a monosemicarbazone. It is reduced catalytically into an alcoholic base, epi-dihydrothebainol, and shows properties quite different from dihydrothebainol. Its dibenzoyl derivative crystallizes as methiodide.

In the following table, the properties of the two pairs of the ketonic and alcoholic bases are compared.

	M. p. of free base	[α] D	M. p. of Semicarbazone	M. p. of Methiodide
(+)-Epidihydrothebainone	130°	+37.3°	205°	253°
(+)-Dihydrothebainone[2]	152°	+59.17°	235°	122°
(+)-Epidihydrothebainol	154°	+48.6°	—	282°
(+)-Dihydrothebainol[3]	165°	+31.5°	—	278°

By the way, we tried the dehydrating action of thionyl chloride on tetrahydrosinomeninone. However, the dehydration did not occur and thionyl chloride formed a cyclic sulfurous acid ester (VI) with the vicinal glycol of the base. This fact coincides well with the investigation of Kitasato and Sone.[4]

The thionyl ester gives the sodium nitro-prusside reaction and regenerates the original base, when warmed with hydrochloric acid, and gives a ferric chloride reaction, thus the phenol group seems to remain intact in the product.

Experimental (By K. M.)

(+)-Epi-dihydro-thebainone (IV). Solution of (+)-tetrahydro-sinomeninone (2 g.) in 55% sulfuric acid (15 c.c.) was boiled for 1.5 hours in an oil-bath. As the concentration of sulfuric acid plays a considerable part on the yield, it should be kept exactly 55%. The cooled solution was diluted with water, made alkaline with caustic and fixed soda, and extracted with chloroform. The chloroform residue was dissolved in acetone and the acetone solution was diluted with ether of its half volume. The precipitate was filtered off and the filtrate was concentrated, dissolved in acetone and rubbed with a glass-rod. The crude base thus crystallized out melted at 120°C. (Yield 0.5 g.). The pure epidihydro-thebainone was obtained through its semicarbazone.

Semicarbazone. To the aqueous solution of the above crude base (4 g.), a sufficient quantity of semicarbazide hydrochloride and sodium acetate was added and the mixture was heated for 15 min. on a steam-bath and left overnight. The semicarbazone was isolated with chloroform and recrystallized from alcohol and acetone. M. p 205°C. Yield 1.6 g. (Found: C, 63.70; H, 7.02; N, 15.79%. Calc. for $C_{19}H_{26}N_4O_3$: C, 63.69; H, 7.26; N, 15.64%.)

Regeneration of the free epi-dihydro-thebainone was effected by boiling the semicarbazone (0.8 g.) with 40% sulfuric acid (20 c.c.) in an oil-bath (130-135°C) for an hour. The free base crystallized out in prisms from acetone. M. p. 130°C. $[\alpha]_D^{10} = +37.3°$ (c=2.200 in chloroform). (Found; C, 72.00; H, 7.455; N, 4.595%. Calc. for $C_{18}H_{23}O_3N$: C, 71.76; H, 7.64, N, 4.65%).

Methiodide. M. p. 253°C from alcohol. (Found: I, 28.59%. Calc. for $C_{18}H_{23}O_3N.ICH_3$: I, 28.70%).

(+)-Epidihydro-thebainol (V). The (+)-epidihydro-thebainone (0.5 g.) was dissolved in methanol (20 c.c.), added with PtO_2 (0.05 g.) and reduced catalytically at 30°C. The

(2) K. Goto and S. Mitsui, This Bulletin, **5** (1930), 282.

(3) Prepared by catalytic reduction in the same way as is shown in the experimental part. These figures coincide well with those given by Skita and others (*Ber.*, **54** (1921), 1560) on (−)-dihydrothebainone, but somewhat differ from those of (+)-dihydrothebainone, prepared by Na-amalgam reduction.[1] A similar case is known in the catalytic reduction of cholestanone (*cis-trans* isomerism of H and OH).

(4) *Ber.*, **64** (1931), 1142.

absorption of hydrogen amounted to 50 c.c. On evaporating the methanolic filtrate, the free base crystallized out in prisms. M. p. 125°C, after sintering at 115°C. However, the dried specimen for analysis melted at 154°C. $[\alpha]_D^{17} = +48.6°$ (C=1.768, chloroform). (Found: C, 71.57; H, 8.345; N, 4.49%. Calc. for $C_{18}H_{23}O_3N$: C, 71.29; H, 8.25; N, 4.62%).

Methiodide. M.p. 282°C from alcohol. (Found: I, 28.88%. Calc. for $C_{18}H_{23}O_3N \cdot ICH_3$: I, 28.54%).

Dibenzoyl-(+)-epidihydro-thebainol-methiodide. (+)-Epidihydro-thebainol (0.1 g.) was dissolved in dry pyridine (1 c.c.) cooled with freezing mixture and added with benzoyl-chloride (0.15 g.). The mixture was left overnight and then decanted into a saturated solution of ammonium carbonate. Two days after, the syruppy precipitate was washed with water and taken up in chloroform. The washed and dried chloroform gave an oily residue when evaporated. The dibenzoyl-derivative was too soluble in organic solvents, such as alcohol and benzene and did not crystallize from ether also. It was, therefore, turned into its methiodide in alcoholic solution. M. p. 273°C recrystallized therefrom. (Found: C, 60.53; H, 5.898; N, 2.14; I, 19.23%. Calc. for $C_{33}H_{36}O_5NI$: C, 60.64; H, 5.51; N, 2.14; I, 19.45%).

Thionyl-ester (VI) of (+) tetrahydro-sinomeninone. Dry (+)-tetrahydro-sinomeninone (1 g.) was dissolved in dry pyridine and the solution was slowly decanted into ice-cooled thionyl chloride (6 g.). After being kept standing two hours, the clear solution was poured on ice, and made alkaline with sodium carbonate. The thionylester was then extracted with chloroform. The dried chloroform was distilled off and the remaining pyridine was removed under diminished pressure. The residue crystallized on addition of methanol. Yield 0.8 g. M.p. 189°C from the same solvent. $[\alpha]_D^{22} = +71.7°$ (C=0.488, in chloroform). (Found: C, 57.27; H, 6.44; N, 3.55; CH_3OH, 7.89%. Calc. for $C_{18}H_{23}NO_5S \cdot CH_3OH$: C, 57.43; H, 6.80; N, 3.52; CH_3OH, 8.06%). The methanol free ester melted at 191°C.

As it has been already shown[5] that (−)-tetrahydrosinomeninone can be prepared from (−)-dihydrothebainone through a series of reactions, it is quite reasonable to assume that (−)-epi-dihydrothebainone and (−)-epi-dihydrothebainol can be prepared in the same way as above. This part of work must be postponed in future for the lack of materials.

Our hearty thanks are due to Mr. Tetsuro Kono and Miss Yosiko Sudzuki for the micro-analysis in this work, and also to Ministry of Education for a grant, which partly defrayed the cost of the present work.

Biochemical Laboratory, Agricultural Department,
Tokyo University.

(5) K. Goto and T. Arai, This Bulletin, **17** (1942), 113.

Fundamental Research on Anodic Reaction. II.* Activation Energy of Anodic Oxygen-producing Reaction on Some Electrode Materials in Aqueous Solution of N/10 Sodium Hydroxide.

By Yukio YONEDA.

(Received July 23, 1949.)

Introduction. The activation energy of electrode reaction can be measured without the knowledge of true area of electrode surface, therefore it is important for the determination of the mechanism of the electrode reaction. Activation energy of anodic exygen-producing reaction in aqueous solution of N/5 H_2SO_4 on platinum was determined by F. P. Bowden[1] to be 13–14 kcals./mole after being corrected by the present author on equilibrium oxygen potential, which will be stated in the present paper. H. P. Stout[2] and nearly at the same time Renè Audubert[3] published the values of activation energy in alkaline solution, but the values calculated were 25.3 and 8.3 kcals. respectively, different from each other. These results are tabulated in the section of "Discussions".

The influence of time on the overvoltage of oxygen anode is remarkable, as Hickling and Hill[4] has stated. Therefore it is important to define the time at which overvoltage is measured. Four different definitions of time are possible as follows:

(a) The time when the maximum curvature on the buit-up curve of potential is obtained.
(b) The time when the potential reaches to stationary state.
(c) The time when the increase of overvoltage per unit time becomes smaller than a certain value.
(d) The fixed time after the closing of electric circuit.

(a) and (b) have some concrete meaning, but (c) and (d) are conventional methods and have rather obscure meaning.

Recently J. N. Agar[5] published his consideration on the theoretical standpoint of activation energy, and classified it into two categories, that is, E_I and E_{II}. E_I is calculated from $\left(\frac{\partial \log i}{\partial(1/T)}\right)_\eta$, taking the temperature coefficient of equilibrium potential into consideration, and E_{II},

* The first paper, *J. Chem. Soc. Japan*, in press.

(1) F. P. Bowden, *Proc. Roy. Soc. London*, A **126** (1929), 107.
(2) H. P. Stout, *Discussions, Faraday Soc.*, No. 1 (1947), 246.
(3) Renè Audubert, *ibid.*, 72.
(4) A. Hickling and S. Hill, *ibid.*, 236.
(5) J. N. Agar, *ibid.*, 81.

from $\left(\frac{\partial \log i}{\partial(1/T)}\right)_V$, neglecting the temperature coefficient, where V, η and i mean the electrode potential, overvoltage and the current density respectively. From the definition of activation energy, E_I is more precise, but it depends upon the value of η. As E_I is equal to $\Delta H^{\ddagger}-\alpha\eta F$, according to Eyring's notation,[6] the present author adopts,

$$\Delta H^{\ddagger}=-2.303\ R\left(\frac{\partial \log i}{\partial\ 1/T)}\right)_{\eta}+\alpha\eta F, \quad \ldots\ldots\ldots(1)$$

as true activation energy which should be independent of η, where F is Faraday constant, α is a constant, and T is absolute temperature.

Experimental Procedures. Anode materials used were platinum, magnetite, or natural graphite. Platinum was brightly polished and its apparent area was 5 sq. cm. Magnetite had been shaped from a large artificial magnetite anode of practical size, and its area was 5 sq. cm. As natural graphite, positive plate of dry cell was used, and its area was 5 sq. cm., too. It consisted of natural graphite sintered with organic binder, and was treated with xylene to extract soluble matter.

Electrolyte was the aqueous solution of N/10 NaOH, which was prepared not to be contaminated with carbonate or chloride. As reference electrode, mercuric oxide electrode with N/10 NaOH solution as electrolyte was employed, therefore liquid potential was always negligible. A fine tip from reference electrode was slightly pressed upon the surface of electrode. Electrolyte was always stirred by and saturated with the stream of oxygen gas.

Two electric circuits, that is, ordinary and oscillographic methods, for measuring overvoltage were prepared. The ordinary one consisted of a potentiometer and a galvanometer. The oscillographic method means that with electromagnetic oscillograph and vacuum tube amplifier. The sensibility of vibrator (Yokogawa D type) is 1 mm./μA, and the total sensibility, when combined with amplifier and dipped in damping oil, is 0.5 mm./mv.

The potentials by ordinary method both on platinum, (i)*, and on magnetite and natural graphite, (iii)*, were measured. The electrolytic cell was in a thermostat, but the reference electrode was in room temperature. The potential differences caused by this temperature difference were specially measured afterwards and these amounts were corrected for the overvoltage value. In the case of oscillographic method on platinum, (ii)*, both electrolytic cell and reference electrode were in a thermostat. As there was no liquid potential between them in both cases, the equilibrium potential of oxygen electrode against mercuric oxide reference electrode is calculated from the free energy difference of the following reaction,

$$HgO\,(s) = Hg\,(l) + 1/2\ O_2\,(g) \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Owing to Lewis and Randall[7], and Shibata and Murata[8], the equilibrium potential of reaction (2) is as follows:

T °C	18	25	35	45	65
$E_T°$ (mv.)	302.7	299.5	293.5	288.0	276.0

The overvoltage is therefore calculated, substracting $E_T°$ from measured potential difference.

Results. Case A. Pt, after 30 minutes from closing of the circuits. (Method (i))*. Overvoltage was measured in the range of temperature of 18° to 65°C, and in the range for current density D_A of 10^{-5}

(6) H. Eyring, et al., "The Theory of Rate Processes", 587 (1941).
(7) Lewis and Randall, "Thermodynamics", 484 (1923).
(8) E. Shibata and F. Murata, *J. Chem. Soc. Japan*, **52** (1931), 393.
* Refer to "Summary".

to 10^{-2} A./cm.^{2} To obtain reproducible results, electrode had been cathodically polarized previously for 2 to 3 minutes at a current density which was the same as that of anodic polarization of former run. The length of time of the cathodic polarization was almost uneffective.

The relation of the increase of potential with time to D_A was contrary to the Hickling and Hill's observation,[9] that is, the potential increase per unit time was the greater in the present case, the higher was the D_A. The mean potential increase per 10 minutes (between 20 minutes to 30 minutes from closing of circuit) were zero and 65 mv. for D_A's of 10^{-5} and 10^{-2} A./cm.^{2}, respectively. Two to ten runs, according to the reproducibility, were carried out in the same condition. Mean values of overvoltage ($\bar{x}$), and their unbiased estimates of variance ($\widehat{\sigma^2_{\bar{x}}}$) of $\bar{x}$ are compiled in Table 1.

Table 1.

Temp. (°C)	A./cm.^{2}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
18	$\bar{x}$ (mv.)	491	668	816	1253*
	u.e.v. (mv.^{2})	446	57	100	157
35	$\bar{x}$	430	620	764	968
	u.e.v.	42	51	36	154
45	$\bar{x}$	361	552	737	904
	u.e.v.	189	194	232	58
65	$\bar{x}$	303	435	670	825
	u.e.v.	836	100	62	0

u.e.v. = Unbiased estimate of variance.

Except the figure with asterisk, they hold the linearity in overvoltage vs. log D_A curves, Fig. 1. Making u. e. v. as weight, the coefficient of the Tafel's equation,

$$\text{overvoltage (mv.)} = a + b \log_{10} D_A \text{ (A./cm.}^2\text{)},$$

is calculated for each case of the above measurements through the method of least square, as Table 2, where the error represents the mean square deviation.

Table 2.

Temp. (°C)	a	b	α
18	1308±50	162.5±14.1	0.356±0.031
35	1292±14	172.2± 3.7	0.355±0.008
45	1271±22	179.4± 2.1	0.352±0.004
65	1223±18	192.1± 5.7	0.349±0.010
		mean.....	0.353±0.017

(9) A. Hickling and S. Hill, *Discussions Faraday Soc.*, No. 1 (1947), 237.

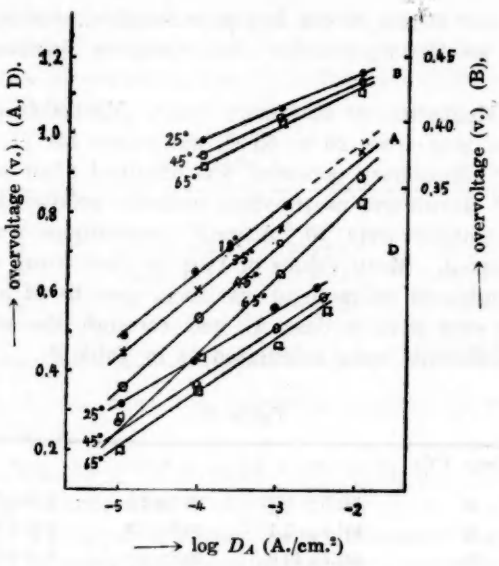


Fig. 1. Overvoltage vs. log D_A.

A: Pt, 30 minutes after closing of the circuit.
B: Magnetite, at the stationary state.
D: Pt, by oscillographic method.

From Fig. 1 and Table 2, we have the following relation at overvoltage of 500 mv., as in Fig. 2,

$$\log D_A = 3.88 - (2.58 \pm 0.41) \times 10^3/T. \quad \ldots\ldots\ldots\ldots (3)$$

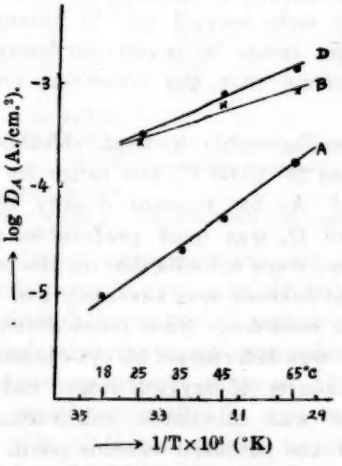


Fig. 2. log D_A vs. 1/T.

From Eq. (1), and taking errors into consideration, activation energy has been found to be 15.9 ± 2.0 kcals. This value is independent of overvoltage.

Case B. Magnetite, at stationary state. (Method (iii)). Temperature range was from 25 to 65°C, and range for D_A was from 10^{-4} to 10^{-2} A./cm.^{2} Stationary potential was obtained after several minutes from closing of circuit and no previous cathodic polarization was carried out. At D_A smaller than 10^{-5} A./cm.^{2}, overvoltage was much lower than that expected. Mean values of two to four runs were plotted in Fig. 1. The unbiased estimate of variance, zero to 42 mv.^{2}, was much smaller in this case than in Case A, and through the above mentioned procedures, coefficients were calculated as in Table 3.

Table 3.

Temp. (°C)	a	b	α
25	492.5± 6.3	26.0±2.5	2.26±0.22
45	491.4± 3.1	28.6±1.3	2.21±0.17
65	491.3±10.0	29.8±4.0	2.25±0.30
			mean.....2.24±0.23

Therefrom we have the following relation at overvoltage of 400 mv., as in Fig. 2,

$$\log D_A = 0.524 - (1.207 \pm 0.086) \times 10^3/T. \quad \ldots\ldots\ldots (4)$$

Thus, activation energy on magnetite was calculated from Eq. (1) as 26.2 ± 3.2 kcals.

Case C. Natural graphite, at stationary state. (Method (iii)). Experiments on graphite were carried out in almost the same method as on magnetite, but the range in which the linear relation of Tafel's equation held was so narrow that the activation energy could not be calculated.

Case D. Pt, by oscillographic method. (Method (ii)). Temperature range was from 25° to 65°C, and range for D_A was from about 10^{-5} to 4.6×10^{-3} A./cm.^{2} As for current density of previous cathodic polarization, one tenth of D_A was most preferable, and variation of its duration, 1 to 3 minutes, were not effective on the potential. After the cathodic polarization, the current was reversed, and the built-up curves of anodic potential were recorded. That point which had the maximum curvature on this curve was determined by eye-measurement. Assuming that the adsorption monolayer of oxygen atoms had been completed at this point, overvoltage was calculated substracting the equilibrium potential, Eq. (2), from the potential of this point. Period of rotation of drum, which was 2 to 6 minutes in these case, was not effective in

determining this point. Mean values of about six runs are plotted in Fig. 1. The unbiased estimates of variance are smaller than 50 mv.^{2}, except a very few anomalous points. Coefficient of Tafel's equation was calculated as in Table 4.

Table 4.

Temp. (°C)	a	b	α
25	925.6± 9.3	120.5±2.8	0.492±0.007
45	902.0±15.3	131.9±4.6	0.479±0.007
65	804.7± 9.2	136.3±2.6	0.492±0.009
			0.448±0.008

From Table 4, we have the following relation at overvoltage of 500 mv., as in Fig. 2,

$$\log D_A = 1.76 - (1.57 \pm 0.11) \times 10^3/T. \quad \ldots\ldots\ldots\ldots (5)$$

Activation energy is therefore, 13.8±0.6 kcals.

Discussions. Values of activation energy of anodic oxygen-producing reaction reported hitherto and those determined newly by the present author are summarized in Table 5. As only E_{II} was to be calculated from the Bowden's experiments, true activation energy defined in Eq. (1)

Table 5.

Researcher	Electrode	Electrolyte	Method	Calculation	$\Delta H^{\ddagger}$ (kcals.)	α	Temp. (°C)
Bowden (1)	Pt	N/5 H_2SO_4	Oscillograph	I*	14.3*	0.5	0–81
					13~14**		
Yoneda	Pt	N/10 NaOH	,,	I	13.8±0.6	0.49	25–65
	,,	,,	(i)	,,	15.9±2.0	0.39	18–65
	Magnetite	,,	(iii)	,,	26.2±3.2	2.24	25–65
Stout (2)	Pt	,,	?	?	25.3	1.0	18–31
Audubert (3)	Pt	N NaOH	?	?	8.3	0.12	?
	,,	0.001 N NaOH	?	?	3.1	0.12	?

* Corrected by the present author.

** Estimated value after taking the liquid potential in consideration.

was re-calculated. After estimating the temperature coefficient of standard single electrode potential of oxygen, $dE_r^0(O_2)/dT$, to be -1 mv./°C, from entropies of hydroxyl ion and so on, and as for that of dissociation constant of water, neglecting the liquid potential between both cells at different temperatures, more correct value of 14.3 kcals. was obtained. Taking the fact into consideration that in acid solution potential of the higher temperature side of liquid contact is less noble than of lower side, more correct value, 13–14 kcals., was obtained.

The Stout's value is too large. The source of this error seems to be that the temperature range in which his experiments were performed was too narrow, and moreover he neglected the influence of time on determining the potential, and it might make α too large, as was pointed out by Bockris.[10]

On the contrary, the Audubert's value of α is too small, and his small value of the activation energy may be led herefrom.

The activation energy on platinum measured by the ordinary method and by osccillographic method agreed with each other. This fact means that there is scarcely any difference between the reaction on so-called monolayer of oxygen atom and that on oxide film which is assumed to be deposited on platinum surface after a certain period of anodic polarization. On the other hand, the discrepancy of value of activation energies and α's between of platinum and of magnetite suggests a discontinuous property between metal and oxide anodes.

Now let us compare the results of the present author in alkaline solution to that of Bowden in acid solution. Method for measurement and calculation are common in both cases. Activation energies and α's are almost the same. Absolute value of overvoltage measured by Bowden, however, is about 250 mv. higher than that by the author, though, according to the former experiment of the author,[11] the overvoltage in aqueous solution of N/10 H_2SO_4 measured by the same method as in Case A was, on the contrary, 100 mv. lower than in alkaline solution. Hoar[12] made an experiment in acid solution by a similiar method to Case A, and his values of 10^{-5} and 10^{-4} A./cm.^{2} are almost the same as the one in alkaline solution above mentioned.

Putting these facts together, the present author suggests the following hypothesis:

In ordinary solution, either acid or alkaline, oxygen is evolved on platinum anode through the same rate-determining step from the same reactant.

Summary.

Activation energies of anodic oxygen-producing reaction on electrode of platinum, magnetite, or natural graphite were measured in aqueous solution of N/10 NaOH under stirring by the stream of oxygen gas, at temperature from about 25° to 65°C. On platinum anode, overvoltage was measured by two methods, (i) by the ordinary method just after 30 minutes from the time of closing the electric circuit, and (ii) by the oscillographic method. The activation energy calculated herefrom was

(10) J. O'M. Bockris, *Discussions Faraday Soc.*, No. 1 (1947), 252.
(11) Y. Yoneda, *J. Chem. Soc. Japan*, in press.
(12) T. P. Hoar, *Proc. Roy. Soc. London*, **A 142** (1933), 628.

found 15.9±2.0 kcals. by (i) method, and 13.8±0.6 kcals. by (ii) method. On magnetite and natural graphite, overvoltage was measured by (iii) ordinary method at the stationary state which was obtained after a few minutes. Activation energy of the former was found to be 26.2±3.2 kcals., and that of the latter is not obtained. The activation energy on platinum by oscillographic method is consistent with that measured by F. P. Bowden in the aqueous solution of N/5 H_2SO_4, which is 13–14 kcals. after being corrected about the temperature coefficient of oxygen equilibrium potential. These facts suggest that the anodic oxygen-producing reaction on platinum proceeds through the same rate-determining step both in acid and alkaline solutions, and the mechanism is rather different on platinum from the one on magnetite.

The author wishes to express his grateful thanks to Professor N. Kameyama and Assistant Professor T. Mukaidou for their kind guidance in this work, and for their kindness in reading the original manuscript.

Department of Applied Chemistry, Faculty of Engineering, Tokyo University.

The Configurations of Δ^5-Cholestenone Oxides.[1]

By Yoshiyuki URUSHIBARA and Misao CHUMAN.

(Received October 28, 1949.)

L. Ruzicka and W. Bosshard[2] were unable to prepare an oxide of Δ^5-cholestenone by oxidation of α-cholesterol oxide with chromic acid, but obtained two stereoisomeric oxides from the action of perbenzoic acid on Δ^5-cholestenone (I). In analogy with cholesterol oxides, they called the compound with higher melting point (m. p. 202°) "α-oxide" and the isomeride with lower melting point (m. p. 122°) "β-oxide". The 5,6-position of the oxido group was demonstrated by transforming the "β-oxide" into the known cholestanedione-(3,6). For the "α-oxide" such an evidence was lacking but the 4,5-position of the oxido group which might result from displacement of the double bond was considered improbable from the fact that Δ^4-cholestenone did not give an oxide with perbenzoic acid.

(1) Abstract from the doctor theses by M. Chuman. Read before the monthly meeting of the Chemical Society of Japan on March 8, 1947.

(2) *Helv. Chim. Acta*, **20** (1937), 244.

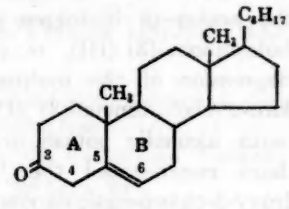
The configurations of cholesterol oxides had already been deduced by one of the present authors,[3] while those of Δ^5-cholestenone oxides remained undetermined. The authors attempted to correlate Δ^5-cholestenone oxides with cholesterol oxides, but, in spite of every effort, the aimed transformation of the former into the latter, or *vice versa*, was not attained. The undesired changes experienced during the unsuccessful attempts will not be recorded in this paper.

In the previous paper[4] one of the authors presented a stereochemical representation of the structures of cholesterol oxides and the courses of the scission reactions of the oxido ring in them. As the structures of Δ^5-cholestenone oxides can be represented quite similarly, it may reasonably be assumed that the rules for the oxido ring rupture of cholesterol oxides hold good as well in the case of Δ^5-cholestenone oxides. Namely, the α-oxido ring is opened between carbon atom 6 and the oxido oxygen atom, while the β-oxido ring between carbon atom 5 and the oxido oxygen atom; the oxygen atom with the hydrogen atom derived from the addenda forms a hydroxyl group with the original configuration; and the residues from the addenda enter the carbon atom cut off from the oxido oxygen atom, all attached to carbon atom 5 taking the α-configuration, thus the β-oxido ring as well as the α-oxido ring producing cholestane derivatives on scission, and certain groups including the hydroxyl group from water and the acetoxy group from acetic acid being determined to assume the β-configuration at carbon atom 6. Now, hydrogen chloride has afforded the key to the determination of the configurations of Δ^5-cholestenone oxides. The configurations of the scission products described below have been inferred from the above-mentioned rules, and the accompanying formulas assume that the chlorine atom entering carbon atom 6 takes the β-configuration like the hydroxyl and the acetoxy groups.

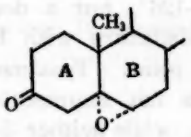
The "β-oxide" of Δ^5-cholestenone gave a scission reaction analogous to that of α-cholesterol oxide: The action of hydrogen chloride on α-cholesterol oxide (V) gave 6-chloro-cholestanediol-(3β,5α) (VI), m.p. 168–169°, which was then oxidized to 5-hydroxy-6-chloro-cholestanone-(3) (III), m. p. 200°, and dehydration of the latter with hydrogen chloride yielded 6-chloro-Δ^4-cholestenone-(3) (IV), m. p. 125–126°. The same 6-chloro-Δ^4-cholestenone-(3) was obtained by the action of hydrogen chloride on the "β-oxide" of Δ^5-cholestenone. In the latter case the intermediate 5-hydroxy-6-chloro-cholestanone-(3) lost water at once in conformity with its structure of a 3-ketone with a tertiary hydroxyl group at carbon atom 5.

(3) Y. Urushibara, This Bulletin, **16** (1941), 182.

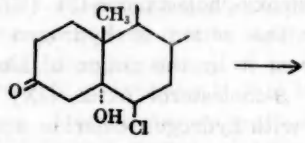
(4) Y. Urushibara, This Bulletin, **22** (1949), 132.



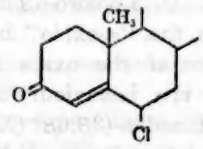
(I) Δ^5-Cholestenone



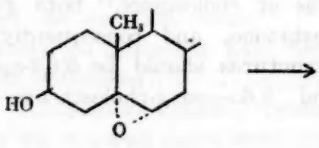
(II) "β-Oxide" of Δ^5-cholestenone



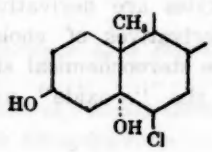
(III) 5-Hydroxy-6-chloro-cholestanone-(3)



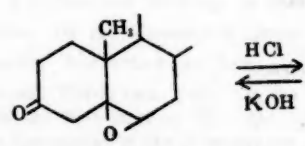
(IV) 6-Chloro-Δ^4-cholestenone-(3)



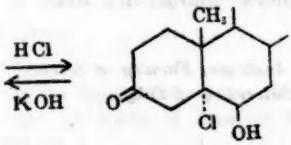
(V) α-Cholesterol oxide.



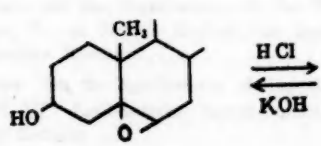
(VI) 6-Chloro-cholestane-diol-(3β,5α)



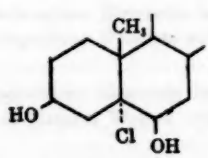
(VII) "α-Oxide" of Δ^5-cholestenone



(VIII) 5-Chloro-6β-hydroxy-cholestanone-(3)



(IX) β-Cholesterol oxide



(X) 5-Chloro-cholestane-diol-(3β,6β)

The "α-oxide" of Δ^5-cholestenone behaves like β-cholesterol oxide: The former gave a product on the action of hydrogen chloride. It differed from 5-hydroxy-6-chloro-cholestanone-(3) (III), m. p. 200°, and melted at 125–126°, but a deep depression of the melting point was observed in admixture with 6-chloro-Δ^4-cholestenone-(3) (IV) with the same melting point. Treatment with alcoholic potash or with many other reagents not enumerated here regenerated the "α-oxide" of Δ^5-cholestenone, while neither 5-hydroxy-6-chloro-cholestanone-(3) (III) nor 6-chloro-Δ^4-cholestenone-(3) (IV) gave either oxide of Δ^5-cholestenone on the action of alcoholic potash or on any other treatment. Hence, the product from the action of hydrogen chloride on the "α-oxide" of Δ^5-cholestenone is 5-chloro-6β-hydroxy-cholestanone-(3) (VIII), and its formation from the "α-oxide" by the action of hydrogen chloride and the regeneration of the oxide from it by the action of alcoholic potash correspond to the behaviour of β-cholesterol oxide (IX) which gives 5-chloro-cholestanediol-(3β,6β) (X) with hydrogen chloride and is regenerated from the latter by the action of alcoholic potash.

The observations outlined in the preceding paragraphs lead to an assignment of a β-oxido (VII) and an α-oxido (II) structures, respectively, to the "α-oxide" and the "β-oxide" of Δ^5-cholestenone. As both cholesterol oxides are derivatives of cholestanol,[4] both Δ^5-cholestenone oxides are derivatives of cholestanone, and consequently the names expressing the stereochemical structures should be 5,6β-epoxy-cholestanone-(3) for the "α-oxide" and 5,6α-epoxy-cholestanone-(3) for the "β-oxide".

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